IMPACTS OF STORM-DRIVEN SURFACE RUNOFF AND LANDSCAPE CHARACTERISTICS ON *SALMONELLA* IN FARM IRRIGATION PONDS IN SOUTH GEORGIA, USA

by

CASEY SHANNON HARRIS

(Under the Direction of George Vellidis and Catherine Pringle)

ABSTRACT

The prevalence of *Salmonella* in waterways of the southeastern U.S. and elsewhere raises questions about the potential role of contaminated agricultural irrigation water in foodborne illness. This thesis provides background on *Salmonella* as a bacterial pathogen and its role in human illness and the environment, and the current status of food safety and related concerns for fresh produce in the U.S. We evaluate the influence of storm precipitation events on *Salmonella* transport through crop fields, forests, and streams in vegetable farm landscapes, and compare *Salmonella* levels in irrigation ponds across southern Georgia in the context of watershed and landscape characteristics.

INDEX WORDS: *Salmonella*, *Escherichia coli*, bacteria, pathogen, water, irrigation, runoff, storm, rainfall, pond, reservoir, agriculture, farm, foodborne illness, MPN, GIS, watershed, land cover

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BS, University of Wisconsin, 2009

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2014

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The University of Georgia
May 2014

DEDICATION

To my family.

ACKNOWLEDGEMENTS

Many people helped make this work possible. Dr. Paige Adams started this research. I did not have the joy of meeting her, but her humor and dedication left a lasting impression on her laboratory co-workers and the farmers she met. I am extremely grateful to Dr. George Vellidis, who dedicated time, resources, and expertise to this research. Each of the members of my committee, Dr. Cathy Pringle, Dr. Karen Levy, Dr. Richard Lowrance, and Dr. Sree Rajeev, assisted in important ways.

Debbie Coker deserves special recognition for teaching me numerous laboratory procedures and dedicating her time to the project without hesitation whenever needed. Camilla Borgato, Debbie Coker, Charles Gruver, Katy Summers, Dr. Moukaram Tertuliano, and Grant Cordle helped analyze water samples for the storm runoff portion of this research, and they made the work a pleasure. In the field, Camilla Borgato assisted with sample collection in the early months of this project. Dr. Lowrance, Dr. Tertuliano, and Marcus Cofield also assisted with sample collection. Herman Henry and Rodney Hill provided technical support, Marissa Grossman provided statistical advice, and Zhiyao Luo provided advice about *Salmonella* analysis. Dr. Rajeev and staff at the UGA Veterinary Laboratory confirmed *Salmonella* positives and shared advice, bench space, materials, and antisera for serotyping. Jill Johnson took the time to teach me PCR and gel electrophoresis techniques. Dr. Adams' lab group collected the water samples reported in the GIS portion of this research in 2011, and Dr. Anita Wright's laboratory analyzed those samples for *Salmonella.* Dr. Taeil Jang and Dr. Nate Nibbelink provided advice on getting started with analyses using GIS software.

Mike Byrne, Scott Connelly, the Conner lab, Ryan Ireland, Lee Kirby, Vasilis Liakos, Andrew Mehring, Marylou Moore, the Pringle lab, Matina Voulgaraki, Jordan West, Aliki Zachou and her friend Dimitris all had a hand in helping me start this research directly or indirectly, sometimes with projects

that did not ultimately make it into this written thesis. Camilla Borgato and Larissa Guimaraes opened their apartments to me for several weeks when I needed somewhere to stay while waiting for unpredictable rainstorms. Family and friends, especially Jack and Daisy, supported me throughout this process.

This work was funded by a grant from the Center for Produce Safety to the University of Georgia titled “Evaluation of sampling protocol to provide science-based metrics for use in identification of *Salmonella* in irrigation water testing programs in mixed produce farms in the Suwannee River watershed” (CDFA SCBGP grant #SCB11063). Other funding was provided by the UGA Graduate School.

Last but not least, I gratefully acknowledge the farmers who allowed me to access their land, when they certainly did not have to do so. They allowed me to set up strange bags, flags, traps, and cameras. They shared great information, advice, and opinions, and helped me realize this research was important and worth pursuing.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

This thesis explores the impacts of storm-driven surface runoff and land cover on *Salmonella* in ponds used to irrigate fruits and vegetables in the southeastern Coastal Plain of the United States. The first chapter provides background on the basic understanding of *Salmonella* as a bacterial pathogen and its role in human illness, as well as the current status of food safety and related concerns for fresh produce in the U.S. and worldwide. The second chapter, "*Salmonella* and *Escherichia coli* in storm-driven surface runoff, streams, and farm irrigation ponds in south Georgia, USA," reports a study of precipitation runoff from agricultural fields and non-agricultural areas into farm ponds. The third chapter, "Impacts of land cover on *Salmonella* concentrations in farm irrigation ponds in rural south Georgia, USA," examines potential correlations between *Salmonella* and the extent of natural or developed land uses at various spatial scales around irrigation ponds. The concluding chapter of this thesis briefly ties together findings and recommendations from both studies.

Foodborne illness in the United States

An estimated one in six people become ill from foodborne illnesses each year in the United States (1, 2). From 1998-2008, approximately 46% of foodborne illnesses were acquired from raw or processed produce, and the rest from meat, poultry, dairy, eggs, fish, or shellfish (3). Half of all foodborne illnesses were attributed to simple products containing ingredients from only one commodity (*e.g.* fish) and the other half to complex products (*e.g.* mixed salad) (3).

The pathogens involved in foodborne illnesses are only verified for about 20% of cases. Of these, more than half are caused by viruses, more than a third by bacteria, and the rest by other organisms (1). In 2011, *Salmonella* caused the largest number of bacterial foodborne illnesses, as well as deaths related

to any foodborne illness (1). *Salmonella* was responsible for over one million foodborne illnesses (1) with an estimated economic cost of $2.7 billion (4). Approximately 1 in every 2,700 foodborne *Salmonella* infections acquired in the United States resulted in death (1).

Other major bacterial pathogens responsible for foodborne illnesses in 2011 (the most recent year for which data are available) in descending order of significance were *Clostridium perfringens*, *Campylobacter* spp., *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp., and *Yersinia enterocolitica* (1). *Listeria monocytogenes* accounted for less than 0.02% of all foodborne illnesses but resulted in a disproportionate 255 deaths (1). In comparison, 378 deaths were attributed to *Salmonella* and 149 to the most common foodborne virus, Norovirus (1). In 2012, foodborne illnesses attributed to bacterial *Campylobacter* spp. (primarily from poultry and dairy) and *Vibrio* spp. (primarily from oysters) were on the rise, while foodborne illnesses from all other causes remained similar to 2006-2011 levels without any statistically significant increase or decrease (5).

Some cases of foodborne illness are connected outbreaks, or situations where more than two people are infected by the same pathogen from the same food or drink. In 2013, the Centers for Disease Control and Prevention (CDC) confirmed 50 multistate outbreaks of foodborne illness (6). Of these, important outbreaks caused by fresh produce in 2013 included *Cyclospora cayetanensis*, a protozoan parasite, in salad mix and fresh cilantro infecting over 630 people in 25 states; hepatitis A virus in pomegranate seeds infecting 162 people; *Salmonella enterica* serotype Saintpaul in cucumbers infecting 84 people; and Shiga toxin-producing *E. coli* O157:H7 in ready-to-eat salads infecting 33 people. Since 2006, major outbreaks of *Salmonella* from fresh produce have included alfalfa sprouts, cantaloupes, mangoes, papayas, and tomatoes. Other major *Salmonella* outbreaks have been attributed to chicken, ground beef, ground turkey, ground tuna, eggs, various frozen products, peanut butter, other nuts, raw cheese, and processed cereals and snacks (6).

Taxonomy of *Salmonella*

Salmonella is a rod-shaped, gram-negative, facultative-anaerobic enteric bacteria. The genus *Salmonella* consists of two species, *S. enterica* and *S. bongori*. Genetic analyses estimate *Salmonella* and its nearest relative, *Escherichia coli*, diverged from a common ancestor 100 million years ago (7), and *S. enterica* and *S. bongori* diverged between 40 and 63 million years ago (8). *S. enterica* is divided into six subspecies (or seven, according to some) based on genetic relatedness (8–10). These six subspecies, as well as *S. bongori*, are traditionally identified in the laboratory by biochemical testing (11).

Salmonella is further divided into serotypes, which are defined by particular combinations of structures known as antigens on the surface of the cell, including portions of the bacterial cell wall, flagella, and capsule. Antigens are structures that induce an immune response in a host. Serotyping is especially important for epidemiological work and can be used to preliminarily identify whether multiple cases of salmonellosis may have come from the same source. Researchers have recognized at least 2,579 unique serotypes of *Salmonella*, defined primarily by combinations of lipopolysaccharide "O" antigens found on the cell wall and flagellar "H" antigens (11). Serotypes of *S. enterica* subsp. *enterica* are given descriptive or geography-themed names (*e.g.* Typhimurium or Tennessee) while all other serotypes are written by their antigenic formula (11). Following important advances in genetic sequencing methods, additional labels are now given to different strains within serotypes to identify clusters of genetic relatedness, *e.g. S. enterica* ser. Typhimurium DT104 (12, 13).

Characteristics of *Salmonella* serotypes

Most *Salmonella* serotypes are considered potentially pathogenic to humans, although the symptoms and severity of infection vary greatly between serotypes as well as between individual people (14, 15). From 2001-2011, over one thousand serotypes among the 2,579 known *Salmonella* serotypes were reported to the CDC as the cause of laboratory-confirmed human illness. Over 300 of these

serotypes were reported in 2011 (16). Overall, however, fewer than 100 serotypes cause the vast majority of human infections (16).

Some *Salmonella* serotypes are host-specific, meaning they only infect a certain type of animal. Typhi infects only humans and causes typhoid fever; Paratyphi A, B, and C primarily infect humans or domestic pets and cause paratyphoid fever (17). A few other notorious serotypes are host-specific, including certain strains of Gallinarum in poultry ("fowl typhoid"), Typhisuis in pigs, Abortusovis in sheep, and Abortusequi in horses (18).

Foodborne salmonellosis, however, is usually caused by nontyphoidal *Salmonella* serotypes. Infections caused by nontyphoidal *Salmonella* serotypes are sometimes zoonotic, meaning the disease is passed between animals and humans. Nontyphoidal *Salmonella* serotypes are often capable of infecting multiple types of animal hosts, including reptiles, amphibians, and birds, as well as humans and other mammals. For *Salmonella*, the path of transmission between animals and humans is often indirect, and the bacteria sometimes survives for considerable amounts of time in water or soil and on other surfaces including food products (19). The long-term survival capabilities of *Salmonella* in environmental and animal reservoirs and the host non-specificity of many serotypes make zoonotic nontyphoidal *Salmonella* difficult to control (19). Typhoid fever, a non-zoonotic and human-specific disease, has become rare in the United States with the advent of ready access to clean water and sanitation systems, while zoonotic foodborne nontyphoidal salmonellosis remains a larger concern (15).

Although most nontyphoidal *Salmonella* serotypes are not host-specific, some common serotype-host associations have been observed. For example, Dublin is primarily associated with cattle and Choleraesuis is primarily associated with pigs, but both are also known to infect humans (18). The majority of human nontyphoidal *Salmonella* infections are caused by serotypes within *S. enterica* subspecies *enterica*, while cold-blooded animals are more commonly associated serotypes within the other five *Salmonella* subspecies. Around 40% of all known *Salmonella* serotypes are considered primarily

associated with reptiles or amphibians and less than 1% of human cases of *Salmonella* infection are caused by those serotypes (20).

The most common *Salmonella* serotypes responsible for human cases of foodborne illness in the U.S. from 2001-2011 were Enteritidis, Typhimurium, Newport, Javiana, Heidelberg, Montevideo, I 4,5,12:i-, Muenchen, Saintpaul, and Oranienburg, with several other serotypes not far behind (16). A study of *Salmonella* outbreaks from 1998-2008 noted some associations between certain serotypes and specific food commodities (21). Enteritidis and Heidelberg caused outbreaks primarily associated with animal-derived foods, especially eggs and poultry, and about 80% of Montevideo outbreaks were also associated with animal-derived foods. Javiana and Saintpaul caused outbreaks more often associated with plant-derived foods. Newport and Typhimurium were associated with a very wide range of food commodities.

Additionally, antibiotic resistance among *Salmonella* is an area of increasing concern. The CDC has identified five serotypes most commonly exhibiting antibiotic resistance to five or more antibiotics commonly used to treat *Salmonella* infections: Enteritidis, Typhimurium, Newport, Heidelberg, and I 4,[5],12:i:- (22).

Transmission of nontyphoidal *Salmonella*: the fecal-oral route

Salmonella cells are typically shed in a host's feces, or other body fluids in severe cases, after successful invasion and replication inside an animal. Some of these bacterial cells may manage to adjust and survive under whatever environmental conditions they encounter outside the host. These conditions include water or soil of varying temperature and pH, plant surfaces or industrial surfaces colonized with competing bacteria, or even suspension in air and exposure to UV rays.

Some *Salmonella* cells eventually manage to enter the mouth of a new host, often carried by some type of contaminated food. Contamination with *Salmonella* may occur at any point in the supply chain through direct or indirect contact with contaminated feces, water, soil, compost, processing

equipment, storage containers, or commercial surfaces. Insects, wildlife, workers, or end consumers may also serve as vectors of *Salmonella* (23). Even the seat of a shopping cart, where shoppers sometimes place fresh produce or raw meat and where young children with non-hermetically sealed diapers may sit, could serve as a two-way transfer point for contamination (24). Various non-food sources of *Salmonella* and other pathogenic fecal bacteria have also been documented, from pet reptiles to holy water (20, 25).

Once inside a host, *Salmonella* cells must withstand acidic conditions in the host's stomach and maintain an intracellular pH around 7.6-7.8 to avoid damage (26, 27). *Salmonella* has mechanisms to cope with moderately low pH environments, but human stomachs may have a pH as low as 1.5, which is severe even for *Salmonella* (28). S*almonella* cells may have a better chance of surviving passage through the human stomach when protected by foods with a high lipid content or other clumps of other *Salmonella* cells (29). When *Salmonella* cells reach the intestine, they must contend with bile salts, low oxygen conditions, and the host's immune response (26). The surviving cells must also compete with the existing gut flora for nutrients and space. Some successful *Salmonella* cells manage to replicate and are shed in the feces of the host to begin the process anew. The host may not necessarily show any symptoms of infection.

In some cases of salmonellosis, especially those involving more host-specific *Salmonella* serotypes, some cells may be ingested by a host's macrophages (18, 30). Activated macrophages are normally part of a host's immune response and help seek and kill invasive organisms, but *Salmonella* inside an inactivated macrophage is protected from the host's immune response and able to multiply. A macrophage may then travel to other parts of the body and spread the infection. The immune status and condition of a host (whether a host has encountered this type of *Salmonella* before and how quickly it can respond) are factors in determining the outcomes of this type of infection (18).

In a study using data from 38 past outbreaks of foodborne *Salmonella*, average ingested doses of 36.3 colony-forming-units (CFU) caused illness with outward symptoms in 50% of the exposed human

population. Less than 7 CFU of *Salmonella* were estimated to cause asymptomatic bacterial replication and shedding in 50% of the exposed human population during the same outbreaks. Higher ingested doses of *Salmonella* tend to increase the likelihood of infection or illness, but the particular condition of an individual person is very important in determining whether the person will become sick. Interestingly, for many *Salmonella* serotypes, the study's models also estimated that a small portion of the exposed population does not develop symptoms of illness even at very high doses (31).

Although *Salmonella* must overcome many substantial barriers in order to cause an infection and many *Salmonella* cells die along the way, it remains a very successful and constantly evolving pathogen. The fecal-oral route, by far the most common transmission route for nontyphoidal *Salmonella* as well as *E. coli*, Norovirus, and many other foodborne pathogens, highlights the need for effective hygiene and safe food preparation.

Global concerns related to *Salmonella*

Widespread complicating health conditions including malnutrition, HIV, and malaria can leave people more susceptible to infection by *Salmonella* and other pathogens. Large highly susceptible populations provide *Salmonella* and other pathogens with ample opportunities to develop increased host-specificity and antibiotic resistance (32). *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis are frequent causes of severe illness and death in parts of sub-Saharan Africa, more than anywhere else in the world (33).

The same serotypes are responsible for about 50% of nontyphodial *Salmonella* infections worldwide, but worldwide Typhimurium and Enteritidis strains are genetically distinct from the most common strains in Africa (34, 35). One of the most common causes of human *Salmonella* infections worldwide since the 1990s is *S. enterica* ser. Typhimurium DT104, which is thought to have emerged in cattle in Europe before its spread to other animals used for intensive food production. In the early 2000s, DT104 also began showing resistance to multiple important antibiotics, now up to seven (33).

Antibiotic resistance will continue to cross international borders by travel and trade. Researchers in Canada recently noticed that an increasing percentage of human isolates of *S. enterica* ser. Kentucky, a relatively rare *Salmonella* serotype, were resistant to ciprofloxacin, an important antibiotic. All Canadian patients infected with serotype Kentucky had actually visited Africa shortly before their symptoms began (36). Canada had previously halted its use of cephalosporin antibiotics, including ciprofloxacin, in livestock to preserve the efficacy of this class of antibiotics in treating humans.

Studies have also linked nontyphoidal salmonellosis with malaria infections, in epidemic proportions (37, 38). Malaria appears to dramatically increase the likelihood of subsequent infection with nontyphoidal salmonellosis. For many people, especially children, overcoming nontyphoidal salmonellosis while also recovering from malaria is a very difficult process with poor survival rates (37, 38). In this way, the global burden of malaria exacerbates the global burden of *Salmonella*.

Efforts toward global health and global economic security are extremely relevant to food safety. The U.S. Food Safety Modernization Act signed into law in 2011 will establish new regulations for imports, but to significantly reduce the burden of foodborne illness in a world with global food trade and travel, producers and consumers everywhere must be able to afford to care about food safety. Ultimately there is no substitute for the effort, time, and expense of food producers intimately understanding their own fields, crops, and packing processes, and for every person involved in the food production chain, including consumers, to be vigilant in carrying out hygiene measures to limit the transmission of our own diseases.

Food Safety Modernization Act of 2011

Under the Food Safety Modernization Act (FSMA), the U.S. Food and Drug Administration (FDA) will be issuing science-based minimum standards for the growing, harvesting, packing, and holding of fruits and vegetables (39), including provisions to ensure the safety of imported food and food during transport. The law aims to reduce the incidence of foodborne illnesses associated with produce

consumption, through the prevention of "reasonably foreseeable" contamination events. Many specific provisions of the law are expected to be finalized in 2015-2016, and will go into effect afterward.

The proposed (not finalized) rules require many growers to monitor irrigation water for microbial contamination, keep records of the results, and ensure the "safe and sanitary quality" of all agricultural water for its intended use. Irrigation water monitoring includes testing for *Escherichia coli*, considered an indicator of fecal pollution. The water testing schedule depends on the type of crop grown, the irrigation method, and the water source. For example, for fruits or vegetables intended for uncooked consumption and grown in direct contact with irrigation water from a pond receiving surface runoff, growers will need to test water once every seven days during the growing season. If the water used is solely ground water, growers will only need to test at the beginning of the season and once every three months. If *E. coli* levels above 235 CFU are present in any 100 mL sample, water use should be discontinued until the grower re-evaluates and re-tests the water source, or unless the grower treats the water to remove contamination.

One concern regarding the water testing portion of FSMA is whether standard *E. coli* tests may adequately predict the likelihood of gastrointestinal illness associated with water usage. Researchers have reported that generic *E. coli* levels poorly predict the presence of more dangerous *E. coli* strains such as *E. coli* O157:H7 in agricultural watersheds (40), and the second chapter of this thesis includes a description of the lack of correlation between *E. coli* levels and *Salmonella* presence in irrigation ponds. Other types of indicators, including total and fecal coliforms, enterococci, *Clostridium perfringens*, and certain bacteriophages, likewise have not been shown to adequately predict the presence of more dangerous pathogens when simply tested alone (41). Overall, a reliance on water testing could provide growers with a false sense of security or unnecessary alarm, yet the implied requirement that growers must physically visit and observe water sources on a regular basis could prove helpful. Another concern regarding FSMA water requirements is insufficient supporting evidence for the use of irrigation water treatment methods including chlorine to improve the microbial quality of water, and the limited availability of other

affordable, efficient, and sustainable water treatment options for agricultural settings (42). Lastly, the time, labor, and costs associated with frequent water testing could discourage growers from using surface water in favor of potentially more limited groundwater resources.

Growers will also be required to ensure the safety, visual quality, and temperature of water sources in contact with produce after harvest. Water for post-harvest use must not contain any detectable *E. coli*. For harvesting, packing, or holding produce, FSMA will require all equipment and materials to be single-use or easy-to-clean. FSMA also sets standards for the use of compost and untreated animal waste. Compost must be treated with appropriate time and temperature measures. Untreated animal waste, if used, must be applied to cropland at least nine months before crop harvest and all direct contact with produce avoided. Growers will be required to monitor fields for animals before and during the growing season, but not required to exclude animals from fields or document their monitoring activities. If a pet or wild animal has been in a field, an unspecified suitable time period must pass before harvest. The law is intended to be compatible with existing natural resource and wildlife conservation practices, and does not recommend the destruction of habitat or the clearing of farm borders around outdoor growing areas or drainages. However, another concern regarding FSMA as currently written is that it does not sufficiently emphasize the need for continued usage of good soil conservation practices, water conservation practices, and wildlife habitat preservation in agricultural settings. The law does not outline the potential environmental impacts of changes in growing practices or long-term effects of disinfectant usage in irrigation water for crops, soil, or the atmosphere.

Many growers have already begun compliance with food safety measures similar to FSMA provisions through voluntary third-party audits (43). For individual farms, the costs of implementing the law are expected to be around $4,000 to $30,000 per year depending on the size of the farm (39). The entirety of the FSMA law is very substantial, and among other education initiatives the FDA and USDA will issue updated guidance manuals for good agricultural practices (44). The FDA estimates 1.75 million of

the total estimated 48 million foodborne illnesses occurring in the U.S. each year will be prevented by the law (39).

Connections between *Salmonella*, animals, and fresh produce production

The global human population has more than doubled in the past 50 years, from 3 billion in the 1960s to over 7 billion today. We live with about 1 billion pigs, 1.5 billion cattle, 2 billion sheep/goats, and 24 billion poultry (45). Massive changes have occurred in global land use as well as the intensity of crop and livestock production, and changes in zoonotic disease transmission have occurred as well.

Salmonella and other pathogens can be transferred between areas of livestock and produce production by a variety of means (46). *Salmonella* may be transported into downstream waterways used for crop irrigation by storm runoff, either through flow paths in soil or surface runoff (47, 48). Pathogens may survive in manure or compost applied to cropland soil (49), and *Salmonella* and other pathogens can also be picked up by wind to travel through the air as bioaerosols (50). A wide variety of wildlife including insects, especially flies, can carry pathogens long distances, as can vehicles, farm equipment, or even workers' boots. The importance of separating produce production from livestock production has been well documented and put into practice by US food producers (51).

Field studies have shown that some pathogens from irrigation water can persist on crop surfaces for several weeks, and other studies have observed *Salmonella* or *E. coli* entrance into plant tissues through wounds or stomata (52, 53). Studies have reported *Salmonella* survival times in soil ranging from a few days to more than 300 days, but studies of root uptake of *Salmonella* or *E. coli* from contaminated soil have produced conflicting results (54).

Humans can also cause contamination at any step in the food supply chain. From planting, harvesting, packing, all the way to the grocery store and consumers' homes, awareness of appropriate hygienic measures is essential. In the pre-harvest environment, there is also potential for human waste to contaminate downstream water sources, especially inadequate sewer and septic systems from nearby

commercial or residential areas, or from landfills or areas where trash is stored (55, 56). Domestic pets including cats, dogs, horses, and backyard chickens are a smaller but recognized risk when in close proximity to food production (57).

While wildlife may certainly transport and spread *Salmonella* and other pathogens, the actual risks are extremely difficult to quantify. *Salmonella* has been isolated from a wide variety of wildlife, with much geographic and seasonal variation, as well as variation in patterns between different *Salmonella* serotypes (58–63). It is difficult to identify actual incidences of animal-human *Salmonella* transmission vs. incidences of human-human transmission, especially when foodborne (64). A survey of California produce growers suggested many have recently felt pressure to implement extreme or even scientifically unsound measures to reduce wildlife presence on farms (65). Wildlife habitat disruption and wild animal exclusion on farms have not been recommended by the U.S. Food and Drug Administration or U.S. Department of Agriculture (39). In some landscapes dominated by agricultural production, the relatively small natural areas that food producers keep on their land are sometimes the only habitats available for wildlife in the area including migratory birds. Natural areas may also provide larger ecosystem services far beyond the context of food safety or wildlife habitat, including flood control, soil conservation, water purification, and even resistance to climate change, in addition to recreational and many intangible benefits (65–67).

Many species of animals and plants have sharply declined amid natural habitat loss. Others, including some considered "nuisance" species by many produce growers and landowners, are thriving in the changed landscapes, accidentally or purposely assisted by us. Those wishing to manage natural areas for particular species of wildlife face a difficult task with often unforeseeable outcomes. For example, well-meaning people led the introduction of Canadian geese to Georgia reservoirs and farm ponds in the 1960s for hunting, and the goose population has since exploded in numbers (68). Geese are now a major concern for some farmers, prompting research into foliar sprays and other methods to deter geese from grazing on crops (69). Geese are one of many species capable of serving as vectors for *Salmonella*, *E. coli*,

and antimicrobial resistance genes (70, 71). The overall importance of wildlife relative to other pathogen sources including livestock waste and human sewage systems is an area requiring more research.

Assessing microbial risks and tracking outbreaks for fresh produce

Quantitative microbial risk assessment (QMRA) is the estimation of public health risk from pathogen exposure. For irrigation water, the purpose of a QMRA would be to determine the relationship between pathogen concentrations in irrigation water and the associated probability of consumers ingesting those pathogens and becoming ill. QMRA, used in reverse, can also be used to determine appropriate standards for water quality. Many variables can be included in a QMRA model - as many variables as researchers are willing/able to measure. At a minimum, the estimates required for an irrigation-related QMRA would be the pathogen concentration in irrigation water, the amount of water applied to a certain amount of produce, the pathogen fraction actually transferred to produce, the fraction surviving until harvest, the fraction surviving until consumption, the amount of produce consumed by a person, the pathogen dose required to infect a certain proportion of the consumer population, and the probability of those consumers becoming ill from a given dose (72, 73).

For *Salmonella*, one study used worst-case-scenario values for QMRA variables to generally estimate the *Salmonella* concentrations required in irrigation water to cause infection in consumers of cantaloupe, lettuce, and bell peppers irrigated by furrow or subsurface drip methods (73). The *Salmonella* concentration required to cause a 1:10,000 rate of illness, the US EPA drinking water standard for contamination, was 2.5 CFU per 100 ml for produce harvested and consumed one day after the last irrigation event, or 5.7×10^3 CFU per 100 ml due to bacterial die-off for produce harvested and consumed 14 days after the last irrigation event (73). The study used a *Salmonella* surrogate, rather than actual *Salmonella.* Considerations involved in specific irrigation QMRAs include the particular pathogens, crops, water source, irrigation method, agricultural practices, environmental conditions, processing/transport conditions, consumer demographics, and much more (72).

Although very difficult, determining QMRAs for various pathogens, crops, and irrigation conditions is possible and could prove to be a useful tool for efficiently and effectively reducing foodborne illness from fresh produce.

Salmonella and other pathogens in surface water used for irrigation

Irrigation and surface water sampling studies in North America have reported *Salmonella* prevalence anywhere from 6% to 100% (48, 71, 74–77). It is not clear whether some regions have consistently greater *Salmonella* prevalence than others; comparisons between regional studies are difficult due to differences in sample volumes and analysis methods. In some regions, including the southeastern US, higher *Salmonella* concentrations have been observed during rainy seasons or warmer months, but the reasons for this seasonality are unclear (72, 78). At smaller spatial scales, higher pathogen concentrations have been associated with sediment and algae, especially near the banks of waterways (79, 80). *Salmonella* and other pathogens are damaged by UV light, and sediment and algae may provide bacteria with nutrients and carbon sources as well as some protection from sunlight. The water management implications of small-scale spatial variations in *Salmonella* concentrations have not been well investigated.

Irrigation water is widely recognized as a potential source of pathogens including *Salmonella*, but only a few outbreaks of foodborne illness in the U.S. have been clearly attributed to contaminated irrigation water (72). The lack of many clear links between foodborne outbreaks and irrigation water may be due to the difficulty of investigating such situations. By the time a particular foodborne outbreak has been recognized, and in the rare situations where the outbreak has been traced to a particular farm rather than another point in the food supply chain, a substantial amount of time has already passed since the crop was potentially irrigated with contaminated water. The presence of particular pathogens in waterways can be temporary and is influenced by flow rates, weather, seasonal patterns, land use, water

management, other human or wildlife activities, and the biology and ecology of different pathogens and the microbial community (72, 75, 78).

Of the outbreaks that have been traced to contaminated irrigation water, an outbreak of *E. coli* O157:H7 from lettuce sold by a popular Midwestern taco chain was likely caused by irrigating with well water mixed with water from a dairy lagoon (81), and another outbreak of lettuce-borne *E. coli* in Sweden was likely caused by irrigating with water downstream from a cattle farm (82). In both cases, the specific outbreak strain, more specific than simply the same serotype, was subsequently isolated from these bovine sources as well as consumers who had been infected. After outbreaks in 2002 and 2005 of *Salmonella enterica* serotype Newport from tomatoes, the outbreak strain was isolated in both years from ponds in Virginia used to irrigate the tomato fields (83).

A few other foodborne outbreaks have been indirectly attributed to contaminated irrigation water. After an outbreak of *S. enterica* ser. Saintpaul from jalapeño peppers and serrano peppers grown by a farm in Mexico, the specific outbreak strain was not isolated from that farm but was isolated from irrigation water at a different farm which provided produce to the same packing facility, perhaps highlighting the importance of packing facility sanitation (84). In an *E. coli* O157:H7 outbreak from baby spinach, the specific outbreak strain was isolated from nearby river water, but the spinach field had been irrigated with well water from a shallow aquifer which did not contain the outbreak strain at the time of sampling, and it is unclear whether river water seeped into the well or another vehicle such as a wild animal transported the pathogen to the field (85).

Several studies have surveyed *Salmonella* in base flow or storm flow in natural waterways in agricultural regions (71, 74, 77, 78, 86), and others have similarly surveyed other pathogens (74, 85, 87, 88). The concentrations of pathogens and indicator organisms in waterways may be elevated following rainstorms, due to surface runoff or subsurface flow into waterways and re-suspension of bacteria from bottom sediments (89). Conversely, depending on land use and sources of bacteria in the landscape,

heavy storms or series of storms may instead dilute pathogen concentrations in waterways (90). Several studies have also addressed the impacts of watershed land-use characteristics on *Salmonella* or other pathogens in irrigation water or natural waterways (71, 74, 77, 87, 88, 91). Some have found higher *Salmonella* prevalence associated with urban land use or areas where livestock is present (71, 74). To our knowledge, however, no previous studies have directly addressed the fate and transport of *Salmonella* in storm runoff into irrigation ponds, especially in rural agricultural settings without major livestock operations nearby. The next chapters of this thesis will focus more narrowly on *Salmonella* in relation to storm runoff and watershed land use surrounding surface water irrigation sources for fruit and vegetable farms in southern Georgia.

CHAPTER 2

SALMONELLA AND *ESCHERICHIA COLI* IN STORM-DRIVEN SURFACE RUNOFF, STREAMS, AND FARM IRRIGATION PONDS IN SOUTH GEORGIA, USA[1]

[1] Harris, C.S., K. Levy, S. Rajeev, C. Pringle, and G. Vellidis. To be submitted to *Applied and Environmental Microbiology*.

Abstract

The prevalence of *Salmonella* in waterways of the southeastern U.S. and elsewhere raises questions about the potential role of contaminated agricultural irrigation water in foodborne illness. To better understand the influence of precipitation events on *Salmonella* transport in vegetable farm environments, surface runoff samples from produce fields and forests surrounding two irrigation ponds in southern Georgia, USA, were collected during twelve storms of varying intensity. Water samples were also collected from the ponds before and after storms, and from streams flowing into the ponds. A total of 127 samples over eight months were analyzed for *Salmonella*, *Escherichia coli*, and total solids (TS). Storm flow in streams had significantly higher *Salmonella* concentrations than surface runoff or pond water before storms, and higher *Salmonella* presence (100%, n=12) than all other locations. TS and *E. coli* were not correlated with *Salmonella* concentrations. The *E. coli* cut-off value of 235 MPN per 100 ml, the proposed limit under the 2011 Food Safety Modernization Act, was a poor predictor of *Salmonella* presence. *Salmonella* was present in 33% (n=24) of pond water samples before storms and 58% (n=24) after storms, and in 38% (n=21) of surface runoff samples from produce fields and 40% (n=30) from forests. At least 18 *Salmonella* serotypes were present, including several commonly implicated in human illness, with the highest diversity observed in samples from streams. Trends in human and wildlife activity during the study are reported. These findings highlight the need for closer evaluation of in-field crop contamination risk factors associated with irrigation water, as well as design and management of irrigation ponds to adequately reduce microbiological hazards.

Introduction

An estimated one in six people become ill from foodborne illnesses each year in the United States (1, 2). Nearly half of all foodborne illnesses are likely acquired from raw or processed produce, and the rest from animal products (3). In fruit and vegetable production environments, irrigation water from surface freshwater sources is recognized as a potential reservoir of pathogens, and a potential vehicle for the transmission of pathogens to plants and soil (72, 92). A few outbreaks of foodborne illness in the U.S. have been linked to contaminated irrigation water from surface freshwater sources, including outbreaks of *Salmonella enterica* serotype Newport from tomatoes grown in Virginia in 2002 and 2005 (81–85).

In recent years, *Salmonella* has caused the largest number of bacterial foodborne illnesses as well as deaths related to any foodborne illness in the U.S. (1). *Salmonella* is commonly present in surface freshwater sources; studies from several agricultural regions of the U.S. have reported detectable *Salmonella* levels in 6% to 100% of surface water samples, although comparisons between studies are complicated due to differences in sample volumes and analysis methods (48, 71, 74–77). A study of ten ponds used for irrigation in south Georgia found *Salmonella* in 11% to 50% of three-liter samples collected monthly from each pond for one year, and the study used sensitive methods capable of detecting *Salmonella* levels as low as 0.055 MPN per 100 ml (93). *Salmonella* levels in irrigation ponds in south Georgia and elsewhere in the U.S. may occasionally exceed thresholds likely to contribute to illness in consumers of fresh produce; a quantitative microbial risk assessment conducted in Arizona using *Salmonella* surrogates in water used to irrigate cantaloupe, lettuce, and bell peppers by furrow or subsurface drip irrigation estimated that *Salmonella* levels of 2.5 MPN per 100 ml in irrigation water could cause illness in 1 per 10,000 consumers, when produce is harvested one day after the last irrigation event followed by typical produce processing procedures (73).

Some *Salmonella* serotypes found in studies of waterways and irrigation ponds in south Georgia and elsewhere in the U.S. are also commonly found in cases of foodborne illness, raising questions about

possible connections between irrigation water and human health (59, 75, 94). The genus *Salmonella* includes more than 2,579 serotypes defined by antigens on the cell surface (11). Most *Salmonella* serotypes infect multiple species of animals, but some serotypes are much more commonly associated with human illness than others (20, 21, 95).

Surface freshwater sources, including irrigation ponds, are critically important for U.S. crop production. An estimated 75 billion gallons of water per day are withdrawn from ponds and streams to irrigate cropland in the U.S. (96). In the southeastern coastal plain of the U.S., the landscape includes hundreds of thousands of small ponds, many of which are used to collect and store surface freshwater for crop irrigation (97, 98). In south Georgia alone, an estimated 160,000 natural or man-made ponds exist along perennial and intermittent streams, and one-fourth of these ponds are located immediately adjacent to farmland (99) (Jim Hook, personal communication). Proposed requirements under the U.S. Food and Drug Administration's Food Safety Modernization Act (FSMA) will hold growers responsible for ensuring the microbial safety of water used for irrigation, necessitating the use of water testing (39, 100). *Escherichia coli* levels above 235 MPN per 100 ml are currently used by growers of fresh produce as a proxy indicator of *Salmonella* (101, 102), but it is far from clear whether *E. coli* levels reliably predict *Salmonella* levels in waterways, especially in the southeast U.S. (74, 103). Unless the proposed requirements are changed, growers using any surface water for irrigation in direct contact with produce intended for raw consumption will be required to test water for *E. coli* once every seven days during the growing season, and discontinue water use if levels exceed 235 MPN per 100 ml in any sample (39).

Some microbes, including *Salmonella*, are capable of persisting in the environment outside animal hosts, and some even live in stable, dividing populations while contending with variations in nutrient availability, temperature, pH, humidity, exposure to UV rays, competing bacteria, and other conditions encountered in the environment (19, 104). Microbes may enter streams and ponds carried by surface runoff during storms or through sub-surface flow paths in soil, although only a few studies have

investigated these pathways specifically for *Salmonella*, and rarely in relation to irrigation ponds (47, 94, 105). Besides direct contact with contaminated irrigation water, potential routes of *Salmonella* introduction to plant surfaces may include improperly composted animal manure, fecal deposition by wild animals, insect contact, or unclean farm equipment (23). Soil splash from rainfall or overhead irrigation may also deposit *Salmonella* from soil onto plant surfaces where *Salmonella* may be capable of adhering (47, 106).

Maintaining vegetated areas around ponds and waterways has long been viewed as a good agricultural practice contributing to soil conservation and improved water quality (107, 108), but some growers of fresh produce have recently converted these areas to bare ground due to concerns about wildlife presence or potentially contaminated runoff from areas of perceived wildlife habitat such as forests adjacent to irrigation ponds (65, 67). We undertook a study to examine storm runoff from agricultural fields and nearby forests as a potential route of *Salmonella* transport into irrigation ponds. We compared the levels of *Salmonella* in surface runoff with levels in storm flow in streams, as well as the levels found in irrigation ponds before and after storms. Additionally, we examined the serotypes found in water samples and monitored wildlife activity at the study sites.

Materials and Methods

The southeastern coastal plain and Little River watershed

The southeastern coastal plain (SECP) is an ecoregion spanning parts of the southeastern U.S. from Louisiana to Virginia (109). The area is important for vegetable crop production, with a long growing season usually allowing two crops per year (110). In the heart of the region, the Little River watershed (LRW) (U.S. Geological Survey HUC-8 03110204) (Figure 2.1) is a 2,311 km^2 area of low topographic relief with broad alluvial floodplains, natural terraces, and gently sloping uplands (111). Soils are primarily sand and sandy loam, shallowly underlain by the near-impermeable Hawthorne formation. The Hawthorne formation results in a dense network of low-gradient stream channels bordered by riparian forest

wetlands (111–113). These riparian areas collect storm runoff and lateral groundwater flow, and serve as buffers to reduce downstream pollution from agricultural runoff (114). Water from the LRW enters the Withlacoochee River, which flows into the Suwannee River and drains into the Gulf of Mexico. The climate of the LRW is humid subtropical, with uneven yearly rainfall distribution often occurring as high-intensity short-duration thunderstorms (110, 115). Federal agencies and researchers have identified the LRW as representative of agricultural practices, climates, and water resources of the SECP (116, 117).

We determined land use statistics for the LRW for this study using QGIS 2.0.1 and publicly available data layers (118–120). In summer 2013, approximately 18% of the watershed's land cover was row crop agriculture including the production of fruits, vegetables, or nuts potentially intended for raw sale. Corn and soybeans together accounted for 46% of row crop acreage, while other crops accounted for the remainder. Data were not available for winter crops.

Study sites

The two ponds in this study were located within the LRW on commercial produce farms. Both ponds were part of a previous 10-pond study of *Salmonella* as well as *Campylobacter jejuni* and *Escherichia coli* O157:H7 in fruit and vegetable irrigation water sources of the SECP (103, 121, 122). Maps of the watersheds and land cover surrounding both ponds were created using QGIS 2.0.1 and the data layers used for the LRW above. The ponds were originally built by damming small, heavily vegetated, slow-moving streams typical of the southeastern coastal plain. Pond 1 drained a 2.8 km^2 watershed and Pond 2 drained a 0.7 km^2 watershed, both heavily agricultural (Table 2.1, Figures 2.2-2.3), and Pond 1 received additional groundwater pumped into the pond to increase irrigation system capacity. Between spring 2012 and summer 2013, crop fields within 250 m of Pond 1 included tomatoes, eggplants, watermelons, jalapeño peppers, and peanuts. Fields within 250 m of Pond 2 included cantaloupes, peanuts, cotton, and a biofuel grass, *Miscanthus giganteus*.

Samples

A total of 127 water samples were collected before, during, and after six storms at each of the two study sites approximately once per month between January and August 2013 (12 total storms) (Table 2.2). Storms were anticipated by following local weather forecasts and weather radars. Only one pond was sampled per storm. Pond water samples were collected before and after each storm to assess any changes in pathogen concentrations occurring as a result of surface runoff, and surface runoff samples from areas of different land uses surrounding the ponds were collected during each storm for comparison. Samples were also collected from streams and ditches entering the ponds.

Pond water samples

Less than 24 hours before each storm, two samples of pond water were collected from the pond edges. Each sample consisted of three sterile disposable 2-L Whirl Pak grab bags (Nasco, Fort Atkinson, WI) totaling 6 L. One sample was collected from the pond edge near the intake pipe for the irrigation system, and the other sample was a composite collected from three separate edges of the pond including the intake pipe location to help characterize the pond as a whole. The same locations were sampled again immediately after each storm or before sunrise the next morning if the storm occurred overnight.

Pond water monthly samples

Additional samples of pond water were collected at the same locations described above. Rather than before or after storms, samples were collected at pre-determined times, usually the second Monday morning of each month. The sampling strategy (one edge vs. three edges) alternated every other month. These samples were collected for a related study and are reported here simply for comparison purposes.

Storm runoff samples

Storm-driven surface runoff was collected during storms from areas surrounding the ponds. Storm runoff collection bags were set up less than 24 hours before each storm. Sampling locations were chosen to represent runoff from each type of land use surrounding the ponds. For each sample, four to

six 2-L Whirl Pak bags were spread out relatively evenly across a 50 to 100 m area parallel to the pond (Figure 2.2-2.3). The bags were propped open with Whirl Pak's built-in wire and pinned securely to the ground with autoclaved nails. The bags were collected immediately after each storm or before sunrise the next morning for overnight storms. The bags were rarely filled completely by storm runoff, and final sample composites totaled 5-7 L per sampling location, depending on the intensity of the storm.

Descriptions of storm runoff sampling locations

At Pond 1, storm runoff sampling locations included three forested areas and two fields. The forested areas included a dam, a mature pine forest bordered by mowed grass, and a forested residential yard also bordered by mowed grass, all sloping toward the pond. Of note, the forested dam doubled as a popular fishing spot for many visitors and the mature pine forest was clear-cut in April. The fields at Pond 1 included a peanut field and tomato field. The peanut field was not planted until July. Plastic mulch and drip tape were installed at the tomato field in January, seedlings were planted in March, tomatoes were harvested in July, and the remaining plant material decomposed in the field in August.

At Pond 2, storm runoff sampling locations included two field areas and two forests. One field area included peanuts and cotton, both planted in June. The other field was a perennial biofuel grass, *Miscanthus giganteus*, harvested in March and allowed to regrow from rhizomes. One forest was a mature pine forest bordered by a wet buffer of sedges and rushes sloping toward the pond. The other forest included young pines (~3-5 years) and shrubs, and was a more upland area that dropped abruptly at the pond edge.

Sampling bags for all runoff locations were set up as close to the pond as possible. The forested sampling locations and peanut field at Pond 1, as well as the *Miscanthus* field at Pond 2, included narrow shrubby/grassy buffer zones 1-3 meters wide at the pond edges. The two forested sampling locations at Pond 2 extended all the way to the edge of the pond. The cotton field at Pond 1 and the tomato field at Pond 2 were farther from the pond edges. Cotton field sample bags were set up on a grassy buffer at the

edge of the field sloping toward the pond. Tomato field samples were collected directly from furrows. We did not initially intend to sample the tomato field, but began collecting samples in April as the downslope forested area was being clear-cut.

Inflow stream samples

Samples were also collected from a major inflow stream and a roadside ditch entering Pond 1, and an intermittent stream entering Pond 2. The ditch and intermittent stream were dry before storms, and samples were collected by pinning Whirl Pak bags to the stream bed or collecting grab samples immediately after storms. An automated sampler was set up at the inflow stream at Pond 1 to collect water at regular intervals from the stream when it rose above base flow. Samples from each location totaled 5-7 L per stream or ditch. We did not initially intend to sample the ditch at Pond 1 or the intermittent stream at Pond 2; sampling began in April-May after witnessing a few storms and recognizing the importance of those locations.

Sample transport and preparation

All samples were collected with sterile materials and techniques and stored on ice in coolers during transport back to the laboratory. In the laboratory, samples were refrigerated and analyses began as soon as possible within 24 hours of sample collection. The multiple Whirl Pak bags collected for each sample were shaken thoroughly and poured into sterile 10-L glass jars to create composite samples. These composite jars were again shaken thoroughly to mix the contents before pouring for further analyses.

Salmonella analysis

The *Most Probable Number* (MPN) enumeration protocol used in this study was developed by Luo (publication under review) and is a variation on standard methods from the U.S. Food and Drug Administration Bacteriological Analytical Manual and method 1682 from U.S. Environmental Protection Agency, using nonselective primary enrichment broth followed by a selective secondary enrichment broth

and plating on a selective indicator agar (123, 124). MPN protocols usually involve serial dilutions of a sample, but the very low amounts of *Salmonella* present in our water samples did not require dilution. Instead we analyzed three replicates of three undiluted sample volumes (500 ml, 100 ml, and 10 ml) for each sample, resulting in a total of nine replicates per composited water sample. These sample volumes were transferred into sterile containers with equal volumes of lactose broth for primary enrichment. After incubation for 24 h at 36°C, each container was shaken thoroughly and 1 ml was transferred into 10 ml of tetrathionate broth for selective enrichment. The inoculated tetrathionate tubes were incubated for 24 h at 36°C, then streaked onto XLT4 agar. After 24 h of incubation again at 36°C, presumptive positive colonies on XLT4 plates were transferred to CHROMagar Salmonella Plus agar and incubated for a final 20-24 h at 36 °C.

To confirm the species identity of presumptive *Salmonella* isolates grown on agar plates, one isolated presumptive positive colony per replicate was chosen preferably from a CHROMagar plate and stored in Luria broth at ambient air temperature for at least 24 h, then analyzed by PCR to confirm *Salmonella* with primers targeting the *invA* gene (125). To prepare isolates for PCR, 1 ml from the Luria tube was concentrated by centrifuging at ≥14000 × g for 3 min. The pellet was then resuspended in 500 µl of sterile molecular grade water, mixed well, and boiled for 10 min before centrifuging again as above. Five microliters of the supernatant was used for PCR, using a commercially available master mix (TaKaRa Ex Taq, Takara Bio Inc., or Promega PCR Master Mix). Thermal cycling was performed at 95°C for 90 s, then 30 cycles of 95°C for 90 s, 50°C for 30 s, and 72°C for 60 s, followed by 72°C for 5 min and holding at 4°C until analysis. The samples were subjected to agarose gel electrophoresis on a 1% gel containing ethidium bromide. Positive *Salmonella* isolates displayed an amplicon of 244 bp. All positive samples were saved in tubes containing a 50/50 mixture of glycerol and Luria broth and kept at -80°C for long-term storage.

All broths and media were prepared from powdered stocks (Difco, Becton Dickinson, Sparks, MD; Remel, Thermo Fisher Scientific, Lenexa, KS; CHROMagar, Paris, France). Positive and negative controls were included throughout the *Salmonella* analysis protocol as appropriate. *Salmonella enterica* ser. Newport was used as a positive control. Estimates of MPN were calculated based on the presence/absence of confirmed *Salmonella* in each of the nine replicates analyzed per composited water sample (126, 127). The lower and upper limits of detection were 0.0548 and 10.99 MPN/100 ml, respectively.

Salmonella serotyping

Two stages were involved in the serotyping process. The first stage was a "preliminary serogrouping" where frozen *Salmonella* isolates were revived and tested with BD Difco brand *Salmonella* O poly antisera. Up to nine isolates had been frozen per sample, one for each PCR-confirmed positive replicate tube in the MPN analysis. In the second stage, up to three isolates from every different O poly group found in each sample were chosen at random and sent to the National Veterinary Services Laboratory (NVSL) in Ames, Iowa, for serotype identification. The "preliminary serogrouping" strategy allowed us to maximize the diversity of isolates sent to the NVSL. However, preliminary serogrouping was only completed for 132 of the 183 isolates frozen in this study; upon attempting to order necessary additional *Salmonella* O poly antisera during the study, we found that all suppliers were experiencing extreme production delays. Fortunately, the preliminary serogroup for one or more isolates in 54 out of the 56 *Salmonella*-positive samples in this study had already been identified, still providing a reasonable overview of the serotypes present.

To revive frozen *Salmonella* isolates, bacteria from each cryotube were transferred to tubes containing 7 ml tetrathionate broth and incubated for 24 h at 36°C followed by 24-48 hours at room temperature. Bacteria from the tetrathionate broth tubes were streaked onto CHROMagar Salmonella Plus agar and incubated for an additional 24 h at 36°C. Single, typical, isolated, presumptive-positive

colonies from each CHROMagar plate were streaked onto Luria agar and incubated overnight to prepare for preliminary serogrouping.

Preliminary serogrouping involved testing each isolate with progressively less common O antigen poly groups until a positive agglutination reaction was observed. We used seven vials of O poly antisera in the following order: Poly B (which contained O antigens for groups C_1, C_2, F, G, H), Poly A (O antigen groups A, B, D, E_1, E_2, E_3, E_4, L), Poly D (O antigen groups P, Q, R, S, T, U), Poly G (O antigen groups 56-61), Poly C (O antigen groups I, J, K, M, N, O), Poly E (O antigen groups V, W, X, Y, Z), and Poly F (O antigen groups 51-55). For these tests, a single drop of antiserum was placed on a sterile glass slide, and a sterile loop was used to mix a small amount of *Salmonella* with the antiserum. After a positive agglutination reaction was observed for a given isolate with a particular antiserum, the isolate was not tested with additional antisera.

After the preliminary serogrouping process, up to three isolates belonging to every O poly group found in each sample were chosen at random. These isolates were restreaked on CHROMagar Salmonella Plus agar and incubated for 24 h at 36°C. Single, typical, isolated, presumptive-positive colonies from each CHROMagar plate were streaked onto tryptic soy broth (TSB) agar slants and promptly sent to the NVSL for final serotyping. A total of 112 isolates were serotyped.

Other parameters and analyses

Escherichia coli MPN/100 ml for each composited water sample was identified using 24-hour Colilert with Quanti-Tray/2000 (IDEXX Laboratories, Westbrook, ME) according to the manufacturer's instructions, currently an approved method under EPA guidelines (128). Total solids (TS) were measured by filtering known volumes of water through 1.5 um filters (Hach, Whatman, Loveland, CO) and baking, desiccating, and weighing according to standard methods (129). Rainfall at each pond was recorded with tipping bucket rain gauges and data loggers (HOBO, Onset, Bourne, MA) installed at each pond for the duration of the project. Water temperature, turbidity, pH, dissolved oxygen, and conductivity were

measured in-pond with a multiparameter sonde (YSI, Xylem, Yellow Springs, OH) at the time of sample collection, only for pond water samples. Other weather data was obtained from the Georgia Automated Environmental Monitoring Network (130).

Statistical analyses

Samples containing no detectable *Salmonella* were assigned a value of 0.0274 MPN/100 ml (half the lower limit of detection) and samples containing *Salmonella* above the upper limit of detection were assigned a value of 10.99 MPN/100 ml (131). Samples containing no detectable *E. coli* were assigned a value of 0.5 MPN/100 ml (half the lower limit of detection for the *E. coli* analysis). Replicates of samples analyzed for *E. coli* were also diluted by known volumes but sometimes exceeded the upper limit of detection even when diluted; these samples were assigned values equal to the upper limit of detection for the method used. *Salmonella*, *E. coli*, and TS values were natural log-transformed to approximate a normal distribution prior to further analyses.

Mixed-effects models are capable of accounting for multiple levels of non-independence in a dataset (132, 133). In our dataset, sources of non-independence between samples included pond, sampling location, and month of collection (Table 2.3). To determine reasonable confidence intervals for differences between each sample type listed in Table 2.2, we evaluated a linear mixed-effects model using the *lmer* program from the *lme4* package (version 1.0-5) for the R Language and Environment for Statistical Computing (version 3.0.2) (134, 135). The model was fit by restricted maximum likelihood, and the *lmer* notation used for the model statement was Outcome = Type + (1|Pond) + (1|Month) + (1|Location). "Type" was defined as a fixed factor and the rest of the variables were defined as random factors with random intercepts (denoted by "1|") but not random slopes. After evaluating the model, 95% confidence intervals for estimates of each sample type were determined using the profile confidence intervals option included in the *lme4* package.

The dataset was evaluated for outliers using the R package *Influence.ME* (136). The most influential data points in the dataset were from the forest on the dam of Pond 1. Two samples from this location exceeded 10.99 MPN/100 ml, the upper limit of detection, and were the only two samples to do so in this study. When this forest was included in our mixed-effects model for *Salmonella*, the model estimates shifted but their significant relationships did not change. The inclusion or exclusion of any other data point or particular sampling location also did not change the outcomes of the model. *Salmonella*, *E. coli*, and TS values for individual samples, organized by sample type prior to the mixed-effects model analyses, are shown in Figures 2.4-2.7.

Wildlife observations

A total of six cameras were installed around the two irrigation ponds in late January 2013 and recorded animal sightings until late August 2013. The cameras were Bushnell brand (Overland Park, MS) "Trophy Cam" models with motion sensors and infrared flash. The cameras were programmed to take a photo every hour and record timestamps, as well as whenever the motion sensor was tripped. Cameras were located at the inflow streams entering each pond, the downstream dams at each pond, and fields or forests near each pond. The dams at both ponds were travel routes for workers and wildlife, as well as popular spots for fishermen and wading birds.

Human and other mammal sightings were defined as individual animals recorded per camera per day. Wading birds sightings were defined as individuals per pond per day. Whenever humans or other mammals were individually identifiable, e.g. bucks vs. does, they were counted as separate individuals. If multiple photos showing the same species were not distinguishable as separate individuals, they were counted conservatively as a single sighting per camera per day. Compared to humans and other mammals, wading birds of the same species were rarely individually identifiable except when multiple birds were present in a photo.

Cameras operated for different numbers of days each month, due to unpredictable differences in battery life and occasionally water or insect damage, and eventually three of the cameras were stolen. To account for these differences in camera operation, animal sightings were reported per "camera effort", with camera effort defined as the total number of days the relevant cameras operated in a given month. Camera effort for wading bird sightings was defined as the number of days at least one camera was operational at each pond. The number of animal sightings per camera effort is reported as animal "activity". Estimating animal activity this way avoids much of the bias caused by cameras operating less consistently in some months than others.

Because most of the camera locations only received certain types of animal traffic, only within-group comparisons of the results are valid; for example, the total number of bird sightings cannot be accurately compared to the total number of human sightings, but the sightings of humans per camera effort in February can be accurately compared to the sightings of humans per camera effort in March.

Results

Presence/absence data

Salmonella was found in every sampling location during at least one storm occurring between January and August 2013. Forty-six percent (58 of 127) of samples collected overall contained detectable *Salmonella*. The two ponds had equal *Salmonella* detection rates for pond water samples, but Pond 1 had higher detection rates than Pond 2 for surface runoff samples. For the twelve storms sampled (six per study site), the 95% confidence interval (based on standard error of proportion) for the proportion of pond water samples testing positive for *Salmonella* was 0.33 ± 0.21 before storms and 0.58 ± 0.22 after storms. The proportion of surface runoff samples testing positive for *Salmonella* was 0.38 ± 0.23 for agricultural fields and 0.40 ± 0.19 for forested areas. At Pond 1, 0.56 ± 0.38 from fields and 0.56 ± 0.27 from forested areas were positive. At Pond 2, 0.25 ± 0.29 from fields and 0.21 ± 0.25 from forests were positive. The proportion of inflow samples testing positive for *Salmonella* at both ponds was 1 ± 0.04, or

100%. The detection rates per storm (rather than per sample, as some locations were occasionally sampled more than once per storm) are shown in Figures 2.2-2.3.

E. coli was detected in 98% of all samples (122 of 125) analyzed for *E. coli*. The three samples without detectable *E. coli* were pond water collected at Pond 2 before and after a storm in May, and pond water collected at Pond 1 before a storm in June. The two samples from Pond 2 without *E. coli* contained detectable *Salmonella*, while the sample from Pond 1 did not.

Concentration data

Two-tailed 95% profile confidence intervals (α=.05) for *Salmonella* concentrations ranged from 0.02-0.08 MPN/100 ml in pond water before storms, 0.05-0.24 MPN/100 ml in pond water after storms, 0.02-0.14 MPN/100 ml in surface runoff from fields and forests, and 0.22-1.24 MPN/100 ml in inflow storm flows (Figure 2.7). *E. coli* ranged from 2-92 MPN/100 ml in pond water, 265-3955 MPN/100 ml in surface runoff, and 284-4279 MPN/100 ml in inflows (Figure 2.8).

Salmonella levels in surface runoff from fields and forests were not significantly different (*i.e.* the model confidence intervals overlapped) from levels found in pond water before or after storms, but *Salmonella* levels in a river, intermittent stream, and ditch flowing into the ponds ("inflows") during storms were significantly higher than levels found in surface runoff. *Salmonella* levels in the inflows were also significantly higher than levels found in pond water before storms. On average, *Salmonella* levels in the inflows were higher than levels found in pond water after storms, but the difference was not quite significant, *i.e.* the confidence intervals overlapped slightly. *Salmonella* levels were near the lower limit of detection, 0.0548 MPN/100 ml, in pond water prior to storm events as well as in surface runoff. On average, *Salmonella* was higher in pond water after storms, though not significantly.

E. coli levels in surface runoff and inflows were significantly higher than levels found in pond water before and after storms, and also significantly higher than 245 MPN/100ml ($p < 0.05$). *E. coli* levels in pond water were significantly lower and below 235 MPN/100 ml ($p < 0.05$), both before and after

storms. No significant differences were seen between *Salmonella* concentrations before vs. after storms or for fields vs. forests. No significant differences were seen between *E. coli* concentrations before vs. after storms or for fields vs. forests, either.

E. coli as an indicator of *Salmonella*

E. coli levels above 235 MPN/100 ml did not predict *Salmonella* presence in pond water or storm runoff samples. Twenty-six out of 60 pond water samples contained detectable *Salmonella* but none of those 26 samples had *E. coli* levels exceeding 235 MPN/100 ml. Four of the 60 pond water samples did have *E. coli* levels exceeding 235 MPN/100 ml, but none of those four contained detectable *Salmonella* (Table 2.4). Overall, *E. coli* levels above 235 MPN/100 ml correctly predicted *Salmonella* presence or absence in only 53% of pond samples and only 56% of surface runoff or inflow stream samples (Table 2.5).

Physiochemical data

Total solids (TS) ranged from 4-50 mg/L in pond water, 165-2962 mg/L in surface runoff, and 28-415 mg/L in inflows (Figure 2.9). While TS in surface runoff was higher than TS in pond water, TS in inflows was not. No significant difference was seen for TS before vs. after storms, or for fields vs. forests.

Water temperatures of pond samples ranged from 12°C to 34°C, increasing steadily from winter to summer months. The pH ranged from 5.8 (February) to 10.0 (May) before storms, and from 5.9 (February) to 10.1 (May) after storms. Conductivity ranged from 0.10 to 0.15 mS/cm at Pond 1 and from 0.05 to 0.08 mS/cm at Pond 2, with little or no differences in water samples collected before or after storms. Dissolved oxygen concentrations at Pond 1 ranged from 8.7 to 11.8 mg/L before storms and 5.3 to 9.9 mg/L after storms, and dissolved oxygen concentrations at Pond 2 ranged from 9.3 to 13.7 mg/L before storms and 7.3 to 14.1 mg/L after storms. For storms occurring overnight, after-storm samples were collected before dawn to avoid possible UV damage to *Salmonella*; dissolved oxygen concentrations usually increase due to algal photosynthesis during the day.

Seasonality and rainfall

Samples collected in January through March at Pond 1 tended to have lower *Salmonella* levels than samples collected in June through August, but the significance of this was not tested and Pond 2 did not appear to have a similar trend (Figure 2.10).

Log-transformed *Salmonella*, *E. coli*, and TS concentrations were not correlated with total rainfall occurring within two or seven days prior to sample collection; none of the Pearson's r values for any sample types exceeded 0.80 (Figures 2.11-2.12). Pearson's r values close to 1 or -1 would have indicated strong linear relationships either positive or negative, respectively, between the variables. Three of the twelve sampled storm events did not have rainfall within seven days prior to "before-storm" sampling; for the rest of the storm events, total local rainfall amounts for the seven days prior ranged from 0.01 to 1.84 inches. Seven of the twelve sampled storm events did not have rainfall within five days prior (five of these were at Pond 2), and eight did not have rainfall within two days prior. Rainfall within two days prior to before-storm sampling ranged from 0.01 to 0.34 inches.

Total rainfall during the twelve sampled storm events ranged from 0.10 to 3.13 inches. None of these storms were extreme; similar storms usually occur every year in south Georgia (137). The heaviest storm, sampled at Pond 1 in August, had a maximum 5-minute intensity of 0.25 inches and a 60-minute intensity of 0.77 inches. Storms more than twice as large occur every other year in south Georgia; storms with a 5-minute intensity of 0.55 inches and a 60-minute intensity of 2.2 inches have a two-year return interval (137).

Serotyping

Preliminary serogrouping revealed high diversity among isolates, even within samples; some individual water samples, especially those from streams, contained *Salmonella* serotypes from up to four different serogroups. Serotyping conducted by the NVSL revealed 18 different serotypes across the 112 isolates sent for testing. Lists of the *Salmonella* serotypes found in each pond and sample type are shown

in Tables 2.6-2.7 along with the months the serotype appeared. Some of the more persistent *Salmonella* serotypes found in repeated months at these two ponds appeared in every sample type (fields, forests, streams, ponds before and after storms). Some *Salmonella* serotypes associated with human illness (such as var. Muenchen and Saintpaul) did appear in these two vegetable farm environments. However, some of the most common *Salmonella* serotypes associated with human illness (including var. Javiana, Enteritidis, Typhimurium, Montevideo, Heidelberg) were not detected at all (Table 2.8). *Salmonella* serotypes detected nation-wide in 2011 in humans, livestock, and other animals, in comparison to serotype strains found in this study, are shown in Table 2.9.

Simpson's index of diversity is defined here as $1 - \sum_{i=1}^{\infty}(^{p_i}/_{p_{total}})^2$, where p_i is the number of isolates of serotype *i* and p_{total} is the total number of isolates of all serotypes found in the category (138). This value reflects the number of serotypes found in a category and the number of isolates of each serotype. Stream samples had the highest Simpson's indices at both ponds. At Pond 1, 77% of serotypes were found in stream samples. At Pond 2, 50% of serotypes were found in stream samples. Overall, serotype diversity per positive sample (S/N) was almost twice as high at Pond 2 compared to Pond 1. This reflects only the isolates we analyzed (Tables 2.6-2.7).

Human and wildlife activity

The wildlife counts and lists presented in this section are far from exhaustive and are by no means official. The information is provided here to generally illustrate the importance of agricultural landscapes as wildlife habitats and often recreational areas for people. These species were not observed in crop fields, but rather nearby or in irrigation ponds (Table 2.10). We believe that the wildlife communities observed at these ponds can be considered typical of south Georgia farms.

Human activity at the ponds rose from March to August, with a sharp temporary drop in July. In August, the cameras recorded an average of 1.7 unique human visitors near the ponds per day, compared to only 0.25 per day in March and 0.07 per day in July (Table 2.11, Figures 2.13a-2.13b). Workers

responsible for irrigation system maintenance frequently visited the ponds, but a large portion of visitors were actually individuals and families visiting the ponds for recreation (fishing, boating, and occasionally hunting) on evenings and weekends. In July, fields near the ponds were fumigated and field gates were locked with signs posted warning visitors to stay out of the area.

Mammal activity at the ponds was higher in February through June compared to July and August. Mammal activity was highest in May, when the cameras recorded an average of 0.86 unique mammals near the ponds per day. In July and August, the cameras recorded only 0.13 unique mammals per day (Figure 2.13c). Common mammals observed included white-tailed deer *(Odocoileus virginianus),* armadillos *(Dasypus novemcinctus)*, red foxes *(Vulpes vulpes)*, domestic dogs *(Canis lupis familiaris)*, raccoons *(Procyon lotor)*, and bobcats *(Lynx rufus)* (Table 2.12). Mammals less commonly observed in photographs included coyotes, hogs, skunks, and domestic cats. The trail cameras were better-suited to capturing relatively large mammals; small mammals including opossums, rabbits, squirrels, mice, rats, and bats were rarely captured in photographs even though such animals were spotted at both ponds during our water sampling visits.

Wading bird activity increased sharply from February to March, and declined from March to August. In March, the cameras recorded an average of 1.77 wading birds per day compared to 0.32 in February and 0.36 in August (Figure 2.13d). The most common bird species observed in direct contact with pond water throughout the study period were herons (especially *Ardea herodias* and *Ardea alba)*, anhingas *(Anhinga anhinga)*, cattle egrets *(Bubulcus ibis)*, ducks *(Anas platyrhynchos* and *Aix sponsa*), and geese *(Branta canadensis)* (Table 2.13). Wading birds seen on single or rare occasions in photographs or in person included a white ibis, glossy ibis, roseate spoonbill, and wood storks. Smaller birds were rarely captured in photographs. Numerous other bird species were observed near the ponds and can be expected to sometimes defecate near the pond, although they were not observed in direct contact with pond water during our water sampling visits.

Reptiles and amphibians including snakes (*Elaphe obsoleta, Elaphe guttata, Nerodia erythrogaster, Lampropeltis getula, Agkistrodon piscivorus,* and once a *Crotalus* sp.), turtles (especially *Trachemys scripta)*, gopher tortoises *(Gopherus polyphemus)*, frogs, toads, and lizards were seen during water sampling visits.

Discussion

The goal of this study was to investigate the transport of *Salmonella* in storm-driven surface runoff through typical southeastern U.S. vegetable farm landscapes, and to investigate *Salmonella* in farm irrigation ponds receiving surface runoff. Numerous other studies have examined *Salmonella* in waterways at base flow and storm flow in rural areas or in irrigation ponds and other surface water in general (60, 71, 74, 75, 77, 78, 86, 93, 94, 103), but none have examined *Salmonella* in surface runoff especially in fresh produce production landscapes. Proposed regulations under the U.S. Food and Drug Administration's Food Safety Modernization Act (FSMA) are aimed at reducing the incidence of foodborne illness, of which *Salmonella* is the most common bacterial cause in the U.S (1, 39). The law will place special emphasis on the safety of water used in fresh produce production, but does not specifically address *Salmonella* in irrigation water.

Contaminated irrigation water has been responsible for some outbreaks of foodborne illness, including *Salmonella,* from fresh produce (83, 139). Wildlife may contribute to the contamination of irrigation water, and some growers of fresh produce have considered removing forested areas or vegetated buffers adjacent to irrigation ponds even though this is not recommended under FSMA (23, 65, 67). Forests and vegetated buffers do provide habitat for wildlife, but also filter and trap contaminated runoff and erosion (107, 108), and the importance of contamination from wildlife sources relative to human-related sources is not known. In this study, the concentrations of *Salmonella* measured in surface runoff from crop fields and forested areas were not significantly different from one another, suggesting

that replacing forested areas with crop fields would not reduce the concentrations of *Salmonella* in surface runoff entering these irrigation ponds.

Salmonella concentrations were elevated in ponds after storm events, although the difference was not statistically significant. Previous studies of waterways in rural south Georgia, upstate New York, and Ontario observed positive correlations between rainfall and elevated concentrations of *Salmonella* in surface water (60, 75, 94), while a study in central Florida noted a lack of correlation (74). Another study in Puerto Rico observed that storms may sometimes result in reduced concentrations of pathogens in surface water (a dilution effect), although the study did not specifically measure *Salmonella* (140). The lack of a strong difference between *Salmonella* concentrations before and after storm events in the present study may have been due to frequent and unusually high rainfall; from January to August 2013, rain in our study area occurred on 101 days and totaled approximately 52 in., compared with 18-37 in. observed for the same months in 2010-2012 (130). It is possible that some samples collected before storm events may have been affected by previous storms; some studies have suggested that bacterial pathogen concentrations in waterways may remain elevated for as many as 5 days following rainfall (141). Additionally, both ponds were located near irrigated crop fields, which drained into the ponds and may have provided a small but steady subsurface flow of excess water and microbes during the growing season.

Storm flow samples from streams and ditches draining into the ponds contained significantly higher *Salmonella* concentrations than surface runoff or pond water before storms, and likely contributed to the higher *Salmonella* concentrations measured in ponds after storms. Storm runoff transports *Salmonella* to streams and ditches, which can serve as a reservoir for *Salmonella* between storm events, and *Salmonella* may also replicate in streams when conditions are favorable (19). Previous studies in south Georgia and central Florida have noted persistent populations of *Salmonella* in streams even at base flow (74, 94, 105). The streams in this study were intermittent or very slow-moving, and often did

not appear to be flowing between storm events. Between storm events, wildlife activity and human-related activity may contribute additional *Salmonella* to these waterways and ponds.

No livestock operations were located in either watershed, but other potential human-related sources of *Salmonella* were present. Numerous studies have documented increased fecal pollution and even increased *Salmonella* concentrations in areas with greater human populations (57, 60, 86, 91, 142–145). Although this study was conducted in a rural area, some of the same human-related factors may affect water quality. In the watershed of Pond 1, some families owned small numbers of domestic pets (dogs, yard chickens, horses), which in larger numbers have been documented to affect water quality (57). Septic tanks were present in the watershed of Pond 1 but were not investigated; previous studies have suggested nutrient-rich effluent from inadequate septic tanks may increase *Salmonella* survival in soil (56, 146). No buildings, residential or commercial, were present in the watershed of Pond 2. Proper bathroom facilities (portable self-contained toilets and wash stations) were brought to the crop fields near both Ponds 1 and 2 whenever groups of workers were present, but other regular visitors to the ponds (primarily recreational fishermen) may have occasionally used the woods outside of the ponds' watersheds. Flies or other wildlife that come into contact with fecal material have the potential to spread any pathogens present (23). Samples from the dam of Pond 1, a popular fishing spot and a well-traveled area for wildlife, had higher *Salmonella* concentrations than any other forested sampling locations in this study.

Frequent testing of irrigation water for *E. coli* is a major requirement proposed under FSMA, but *E. coli* may not be a good indicator of *Salmonella* levels in irrigation water. The combined correct positive + correct negative rate, 53%, for *Salmonella* presence or absence in water samples based on *E. coli* levels above or below 235 MPN/100 ml in this study, was hardly above a rate that might be achieved by random chance. Conclusions of other studies of southeastern waterways have also been uncertain about the suitability of *E. coli* levels as a proxy indicator for *Salmonella* (74, 93).

Salmonella concentrations present in water samples were not correlated with total solids, even though a high prevalence of *Salmonella* can be found in stream and pond bottom sediments, and wind and water turbulence during storms may re-suspend sediments and associated bacteria (19, 88, 93). Both ponds may have been deep enough to avoid major sediment re-suspension during storms. High levels of total solids do not appear to be a requirement for the presence of *Salmonella* in the water column; a study of ponds, creeks, rivers, and canals in central Florida found *Salmonella* in 100% of 202 concentrated 10-L water samples collected over 12 months, all from rural areas away from animal agriculture, all collected without disturbing bottom sediments, and all with low levels of total solids (74).

The diversity of serotypes found in our study, 18 serotypes among 57 positive samples, was similar to the diversity on a per-sample basis found by studies of waterways in other regions, although the most common serotypes differed. These differences may reflect different environmental adaptations among serotypes in different climates, an area of study that has not yet been investigated. The most common serotypes found in this study were Muenchen, Bareilly, Saintpaul, Rubislaw, III 60:r:e,n,x,z15, Gaminara, and I 38:k:-. A study of a fresh produce production region in California found 16 serotypes among 55 positive samples, most commonly Typhimurium and Give (77). A study of fresh produce production landscapes in New York found 7 serotypes among 26 positive samples, most commonly Cerro, Newport, and Thompson (75). A study of urban and rural waterways Ontario found 38 serotypes among 91 positive samples, most commonly Heidelberg and Typhimurium (60).

Within geographic regions, some *Salmonella* serotypes may be persistent in the environment. In 2005, a study of streams in south Georgia found 13 serotypes, most commonly Muenchen, Rubislaw, and subspecies III serotypes (71). In 2007, another study of streams in south Georgia found 15 serotypes, most commonly Braenderup, Bareilly, Muenchen, Kentucky, and subspecies III (94). A sampling of wildlife and pond water in south Georgia a few years prior to our study found 14 serotypes, most commonly

Muenchen and Montevideo (147). In our study, Muenchen, Bareilly, Rubislaw, Braenderup, and subspecies III were also found, although Montevideo and Kentucky were not found at all.

Many *Salmonella* serotypes found in waterways are commonly isolated from cases of human illness, but it remains unclear to what extent these waterways contribute to the incidence of foodborne illness rather than illness acquired through other environmental exposures. Some of the serotypes most commonly isolated from cases of human and animal illness, including Typhimurium, Heidelberg, Enteritidis, and I 4,[5],12:i- were not found at all in our study and have rarely been found in previous studies of south Georgia waterways (71). Other serotypes commonly isolated from cases of human and animal illness were found in our study and in previous studies in south Georgia. Muenchen, Bareilly, Saintpaul, Rubislaw, and Gaminara were isolated in our study and during previous studies in south Georgia; all five of these serotypes are commonly isolated from cases of human and animal illness in the U.S. and have occasionally been associated with *Salmonella* outbreaks, sometimes from fresh produce and sometimes from animal products or even pet reptiles (148–151).

Conclusions

Salmonella seems to be nearly ubiquitous in environmental waters, although usually in low concentrations. *Salmonella* presence can be expected in irrigation ponds connected to natural waterways, transported to ponds by stream flow or storm-driven surface runoff and remaining in pond bottom sediments for extended time periods. Important questions now facing farmers, regulators, and consumers of fresh produce are related to how, exactly, *Salmonella* is transferred between waterways and various crops, and what risk this carries for consumers. The potential for crop contamination caused by *Salmonella* from waterways is thought to be concentration-dependent, at least in part (73). Future studies may determine minimum *Salmonella* concentrations likely to lead to consumer illness for various crops, regions, and irrigation regimes (73). This type of information is essential for regulators wishing to establish reasonable targets for irrigation water quality, especially if existing water quality standards

based on *E. coli* concentrations do not adequately identify *Salmonella* risks. In the meantime, it is important to avoid pressuring farmers to adopt excessive contamination-prevention strategies that have not been supported by science-based evidence or that conflict with long-standing and well-researched conservation practices, such as removing vegetated buffers around waterways, vegetated borders around fields, or forested areas near ponds.

Tables

Table 2.1

Pond size, watershed size, and land cover characteristics. Percent cover by various land use types was calculated for land within a 250 m radius of each pond edge.

Pond	Pond Area (m^2)	Pond Area (ac)	Watershed Area (m^2)	Watershed Area (ac)	Cropland (%)	Forest / Wetland (%)	Other/ Mowed (%)	Water (%)	Paved (%)
Pond 1	79,935	20.0	2,745,691	686.4	42.9	40.4	15.9	0.0	0.8
Pond 2	46,722	11.7	658,244	164.6	36.8	53.8	9.4	0.0	0.0

Table 2.2
Descriptions of sample types and sample locations. Occasionally more than one sample was collected per location per month.

Sample Type		Pond 1		Pond 2	
		Location	Months sampled	Location	Months sampled
Pond before storms	Pond water collected during dry periods, a few hours before expected storms	Near intake	6	Near intake	6
		Pond edges	6	Pond edges	6
Pond after storms	Pond water collected immediately following storms	Near intake	6	Near intake	6
		Pond edges	6	Pond edges	6
Pond monthly	Pond water collected at regular monthly intervals, regardless of rainfall	Near intake (alternate months)	4	Near intake (alternate months)	4
		Pond edges (alternate months)	4	Pond edges (alternate months)	4
Inflow streams	Water collected from streams or ditches flowing into ponds during storms	Primary stream	6	Stream	3
		Large ditch next to paved road	3	-	-
Fields	Runoff collected at the interface between agricultural fields and ponds during storms	Peanut field	6	Biofuel field	6
		Tomato field	3	Peanut/Cotton fields	4
Forests	Runoff collected at the interface between non-agricultural land and ponds	Residential home with pines	5	Shrubland	6
		Forest	6	Forest	6
		Forested pond dam	5	-	-

Table 2.3
Linear mixed-effect model specification for the *lme4* package. The model was fit by a restricted maximum likelihood method. Model statement for *lmer*: Salmonella = Type + (1|Pond) + (1|Month) + (1|Location).

Variable	Variable type	Levels	Transformation	Description of variable
Type	Fixed factor	6	-	Identifies the sample type (Fields, Forests, etc.)
Pond	Random factor*	2	-	Identifies sample from Pond 1 or Pond 2
Month	Random factor*	6	-	Date range (out of 6 full sampling cycles) of sample collection
Location	Random factor*	24	-	Identifies specific locations of repeated sampling
Salmonella, E. coli, or TS	Outcome		natural log	*Salmonella*, *E. coli*, or TS present in each sample

*Random factors were defined with random intercepts [(1|…) in *lmer* notation], but not random slopes.

Table 2.4
Using *E. coli* samples above 235 MPN/100mL to predict *Salmonella* presence.

E. coli Threshold (235 MPN/100mL)	*Salmonella*		
	Present	Absent	Total
Runoff Samples (includes fields, forests, and inflow streams)			
Above	26	22	48
Below	5	8	13
Pond Samples (includes before/after precip. and monthly)			
Above	0	4	4
Below	26	34	60

Table 2.5
Using *E. coli* samples above 235 MPN/100 ML to predict *Salmonella* presence – percentages.

E. coli Prediction of *Salmonella* Presence	Sample Type		All (%)
	Pond (%)	Runoff (%)	
Correctly positive	0	43	21
Correctly negative	53	13	34
Incorrectly positive	6	36	21
Incorrectly negative	41	8	25

Table 2.6 Serotyping results for Pond 1. S/N is the number of serotypes divided by the number of positive samples in each category. The Simpson's index reflects the likelihood of finding two different serotypes in a random sample of two isolates.

	Storm flow samples			Pond samples			
	Fields	Forests	Streams[1]	Before	After	Total	
Samples	9	16	11	12	12	60	
Positive samples (N)	5	9	11	4	7	35	
Isolates	11	39	47	6	26	129	
Isolates serotyped	10	22	31	6	8	77	Prop. of
Serotypes (S)	5	6	10	5	5	13	isolates
Anatum			Mar				0.03
Bareilly	Aug	Apr Jul	Feb Apr	Jul			0.10
Braenderup			Jun				0.04
I_38:k:-	Apr	Feb	Mar Aug	Jun	Mar		0.09
I_6,7:-:e,n,z15			Aug				0.01
III_16:z10:e,n,x,z15			May				0.01
III_60:r:-				Apr			0.01
III_60:r:e,n,x,z15			Feb Apr Jul Aug		Aug		0.08
Inverness		Jul Aug					0.08
Muenchen	Aug	Aug(3)	Feb Apr Aug		Jul		0.16
Newport	Aug						0.01
Rubislaw		Jul	Feb Mar Jun Jul	Aug	Apr		0.14
Saintpaul	Jun Jul	Mar Aug(2)	Apr May Jun Aug	Aug	Jun(2) Aug		0.23
S/N	1.00	0.67	1.00	1.25	0.71	0.37	
Prop. total serotypes	0.38	0.46	0.77	0.38	0.38		
Simpson's index	0.68	0.80	0.87	0.75	0.78		

[1]Includes two stream samples collected at base flow in May that were not included in the rest of this study – these samples included two isolates of serotypes Saintpaul and III_16:z10:e,n,x,,z15.

Table 2.7

Serotyping results for Pond 2. S/N is the number of serotypes divided by the number of positive samples in each category. The Simpson's index reflects the likelihood of finding two different serotypes in a random sample of two isolates.

	Storm flow samples			Pond samples			
	Fields	Forests	Stream	Before	After	Total	
Samples	12	14	3	12	12	53	
Positive samples (N)	3	2	3	4	7	19	
Isolates	3	9	14	7	20	53	
Isolates serotyped	3	4	11	6	11	35	Prop. of
Serotypes (S)	3	2	6	4	5	12	isolates
Bareilly	Jun				Jun		0.09
Braenderup		Jun					0.03
Gaminara		Jun	Jul	Jun	May(2)		0.29
Give_var._15+			May				0.03
I_38:k:-				Jan			0.03
III_50:nonmotile					Jul		0.03
III_50:r:-			May				0.06
III_60:r:e,n,x,z15				Apr	Apr(2)		0.17
Meleagridis	May						0.03
Muenchen	Jul		Jun	May	Jan May		0.17
Rubislaw			Jun				0.03
Saintpaul			Jun				0.06
S/N	1.00	1.00	2.00	1.00	0.71	0.63	
Prop. total serotypes	0.25	0.17	0.50	0.33	0.42		
Simpson's index	0.67	0.38	0.81	0.78	0.67		

Table 2.8

Comparison of study serotypes with *Salmonella* serotypes found in human clinical cases. Serotypes found in the present study (denoted in **bold**) are shown alongside *Salmonella* serotypes reported from human clinical cases in Georgia, USA, in 2011 (the most recent year of data available – this study was conducted in 2013). Numbers of clinical cases were summarized from CDC data (16).

	Number of human clinical cases in Georgia, 2011	
Javiana	497	
Newport	452	
Enteritidis	215	
Typhimurium	135	
I 13,23:b:-	116	
Muenchen	111	
Typhimurium var. 5-	75	
Montevideo	67	
I 4,[5],12:i:-	65	
Saintpaul	51	
Bareilly	45	Serotypes causing more than 20 human clinical cases in Georgia in 2011.
Braenderup	40	
Mississippi	31	
Rubislaw	28	
Heidelberg	27	
Oranienburg	24	
Agona	20	
Infantis	20	
Anatum	11	
Inverness	11	Rarer serotypes found in the present study.
Gaminara	6	
Meleagridis	1	
IIIb 60:r:e,n,x,z15	0	
I 38:k:-	0	
Give var. 15+	0	
I 6,7:-:e,n,z15	0	
III 60:r:-	0	
III 50:nonmotile	14	(general category for all rough, mucoid, and/or nonmotile)
III 50:r:-	0	
IIIb 16:z10:e,n,z15	0	

Table 2.9

Types of animals known to carry the *Salmonella* serotypes found in this study. Animal data is summarized from 2011 CDC data from National Veterinary Serotyping Laboratories (NVSL), and should not be considered a complete list of all animals susceptible to each serotype (16). Hum.=Human, Rep.=Reptile, Dom.=Domestic.

	Hum.	Rep.	Birds/ Wild	Chicken	Turkey	Dom.	Bovine	Porcine	Equine
Saintpaul	x		x	x	x	x	x	x	x
Muenchen	x		x	x	x	x	x	x	x
Bareilly	x			x	x	x	x		x
Rubislaw	x		x	x		x			x
IIIb 60:r:e,n,x,z15	x								
I 38:k:-	x								
Gaminara	x	x		x		x			x
Braenderup	x		x	x		x	x	x	x
Inverness	x			x					x
Newport	x	x	x	x	x	x	x	x	x
Give var. 15+	x		x	x			x	x	x
I 6,7:-:e,n,z15	x			x	x				
Meleagridis	x		x			x	x	x	x
Anatum	x	x	x	x	x	x	x	x	x
III 60:r:-	--none tested by NVSL--								
III 50:nonmotile	--none tested by NVSL--								
III 50:r:-	--none tested by NVSL--								
IIIb 16:z10:e,n,z15				x					

Table 2.10
Descriptions of wildlife camera locations.

	types of animals observed			
Camera locations	Humans	Mammals	Wading birds	**Working condition**
Pond 1 dam	x	x	x	operated throughout study
Pond 1 forest	x	x	x	damaged in April, then fixed
Pond 1 inflow	x		x	lost around mid-May
Pond 2 dam	x	x	x	operated throughout study
Pond 2 forest/field		x		damaged in April, lost around July
Pond 2 inflow		x		damaged in June, lost around July

Table 2.11
Wildlife activity by month. Sightings (number of days animals were recorded), camera effort (number of days cameras were actually working), and total activity (sightings divided by camera effort) are shown per month for each animal category. Number of sightings can exceed camera effort when multiple individually identifiable animals were recorded per day.

	Mammals			**Humans**			**Wading birds**		
	Sightings	Camera effort	Total activity	Sightings	Camera effort	Total activity	Sightings	Camera effort	Total activity
Feb	36	68	0.53	31	62	0.50	13	41	0.32
Mar	29	52	0.56	15	60	0.25	62	35	1.77
Apr	42	66	0.47	49	90	0.54	65	60	1.08
May	87	101	0.86	71	86	0.83	59	62	0.95
Jun	36	81	0.44	73	74	0.99	39	60	0.65
Jul	6	45	0.13	3	45	0.07	24	34	0.71
Aug	6	47	0.13	80	47	1.70	15	42	0.36

Table 2.12

Common types of waterfowl recorded by month. Wading birds which could not be specifically identified but appeared different from other birds seen the same day were listed as "other wading".

	Camera effort	Great Blue Heron		Great Egret		Anhinga		Other Wading		Cattle Egret		Ducks		Geese	
		Sightings	Activity	Sightings	Activity	Sightings	Activity	Sightings	Activity	Sightings	Activity	Sightings	Activity	Sightings	Activity
Feb	41	5	0.12	8	0.20	0	0.00	5	0.12	0	0.00	21	0.51	0	0.00
Mar	35	10	0.29	15	0.43	3	0.09	6	0.17	0	0.00	21	0.60	2	0.06
Apr	60	17	0.28	28	0.47	2	0.03	4	0.07	0	0.00	4	0.07	9	0.15
May	62	13	0.21	28	0.45	0	0.00	2	0.03	9	0.15	0	0.00	0	0.00
Jun	60	26	0.43	8	0.13	0	0.00	4	0.07	0	0.00	0	0.00	2	0.03
Jul	34	23	0.68	0	0.00	0	0.00	1	0.03	0	0.00	0	0.00	0	0.00
Aug	42	4	0.10	4	0.10	3	0.07	4	0.10	0	0.00	0	0.00	0	0.00

Table 2.13

Common types of mammals recorded by month. Any mammal with 5 or fewer total sightings is listed as "other", along with any mammals that could not be specifically identified but were distinguishable from other mammals seen on the same day.

	Camera effort	Deer		Armadillo		Fox		Dog		Raccoon		Bobcat		Other	
		Sightings	Activity	Sightings	Activity	Sightings	Activity	Sightings	Activity	Sightings	Activity	Sightings	Activity	Sightings	Activity
Feb	68	8	0.12	4	0.06	6	0.09	0	0.00	5	0.07	5	0.07	8	0.12
Mar	52	5	0.10	3	0.06	7	0.13	2	0.04	1	0.02	3	0.06	8	0.15
Apr	66	13	0.20	14	0.21	8	0.12	3	0.05	0	0.00	0	0.00	4	0.06
May	101	61	0.60	7	0.07	2	0.02	6	0.06	3	0.03	0	0.00	8	0.08
Jun	81	18	0.22	1	0.01	0	0.00	8	0.10	1	0.01	0	0.00	8	0.10
Jul	45	1	0.02	2	0.04	0	0.00	1	0.02	1	0.02	0	0.00	1	0.02
Aug	47	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	6	0.13

Figures

Figure 2.1

Location of Little River watershed. Little River flows into the Withlacoochee River, which flows into the Suwannee River.

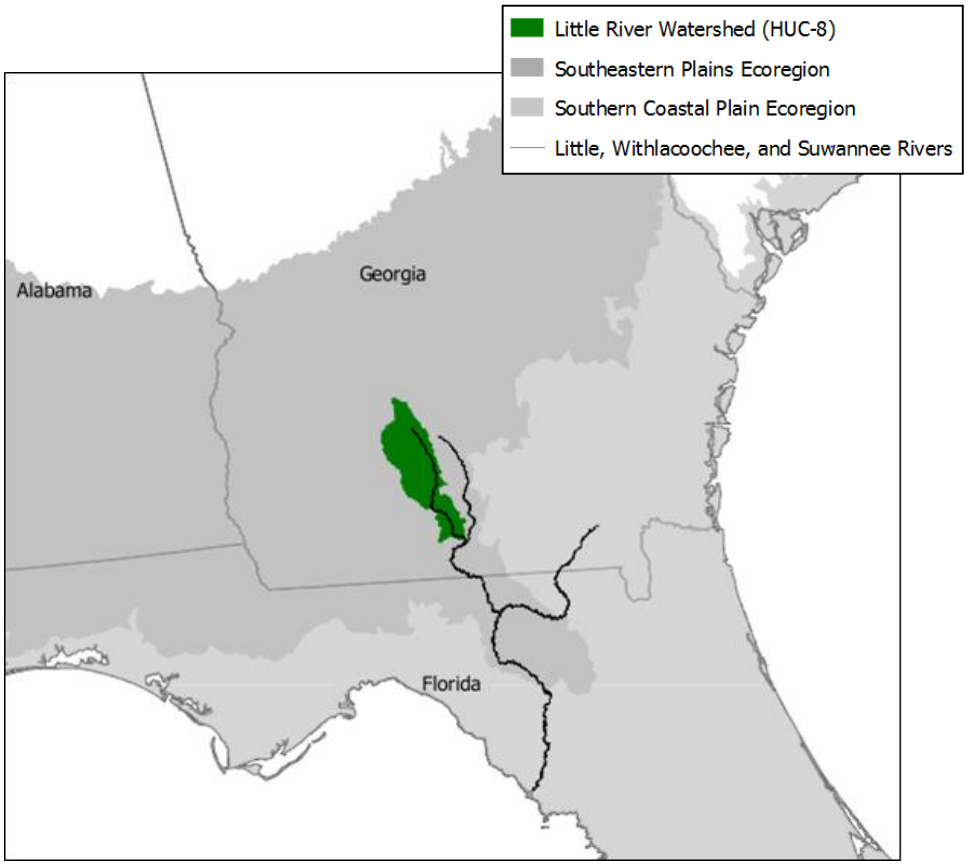


Figure 2.2

Salmonella presence in pond water, streams, and surface runoff at Pond 1. Shown with the percentage and number of storms events during which each location tested positive for *Salmonella.*

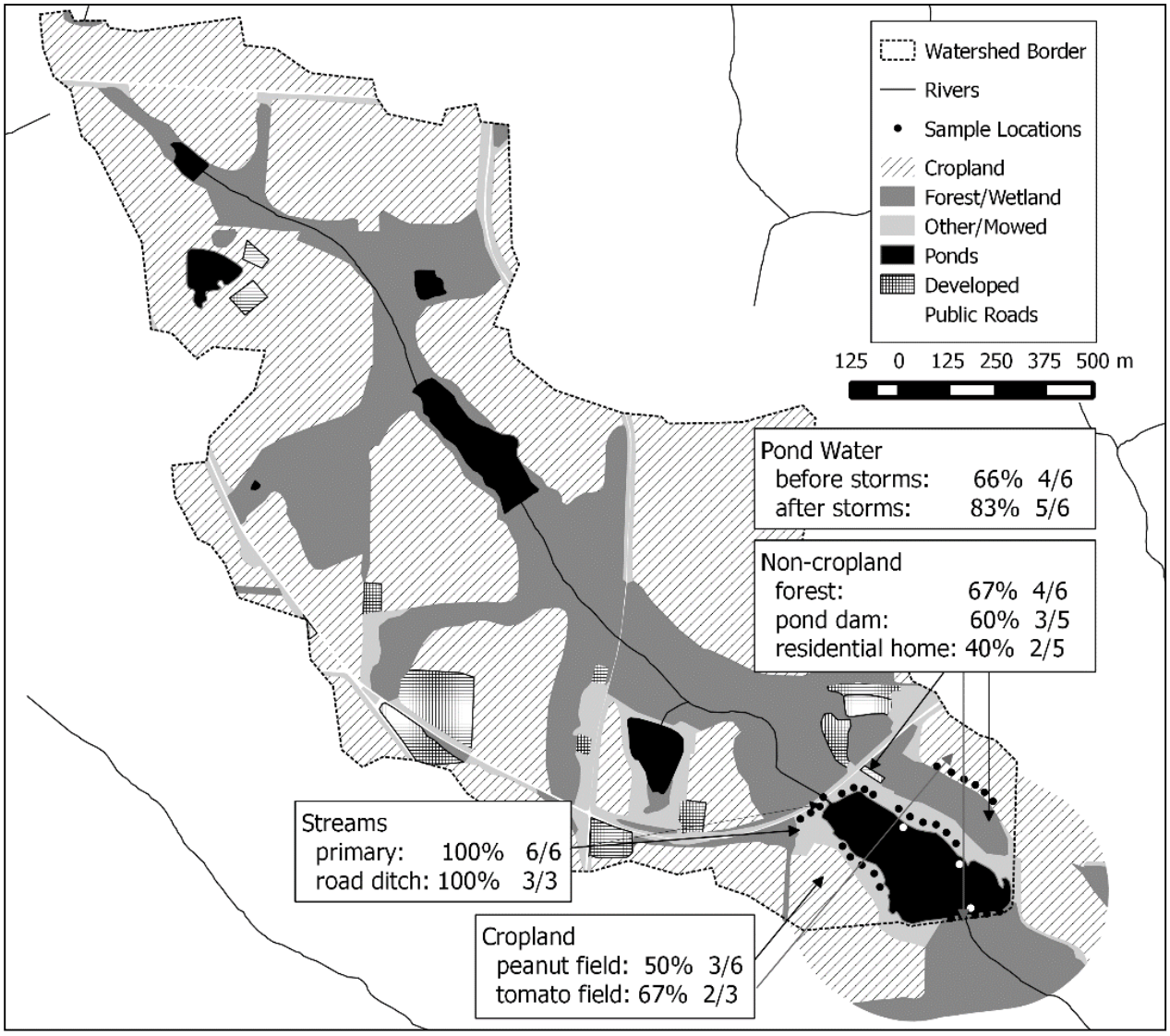


Figure 2.3
Salmonella presence in pond water, streams, and surface runoff at Pond 2. Shown with the percentage and number of storms events during which each location tested positive for *Salmonella.*

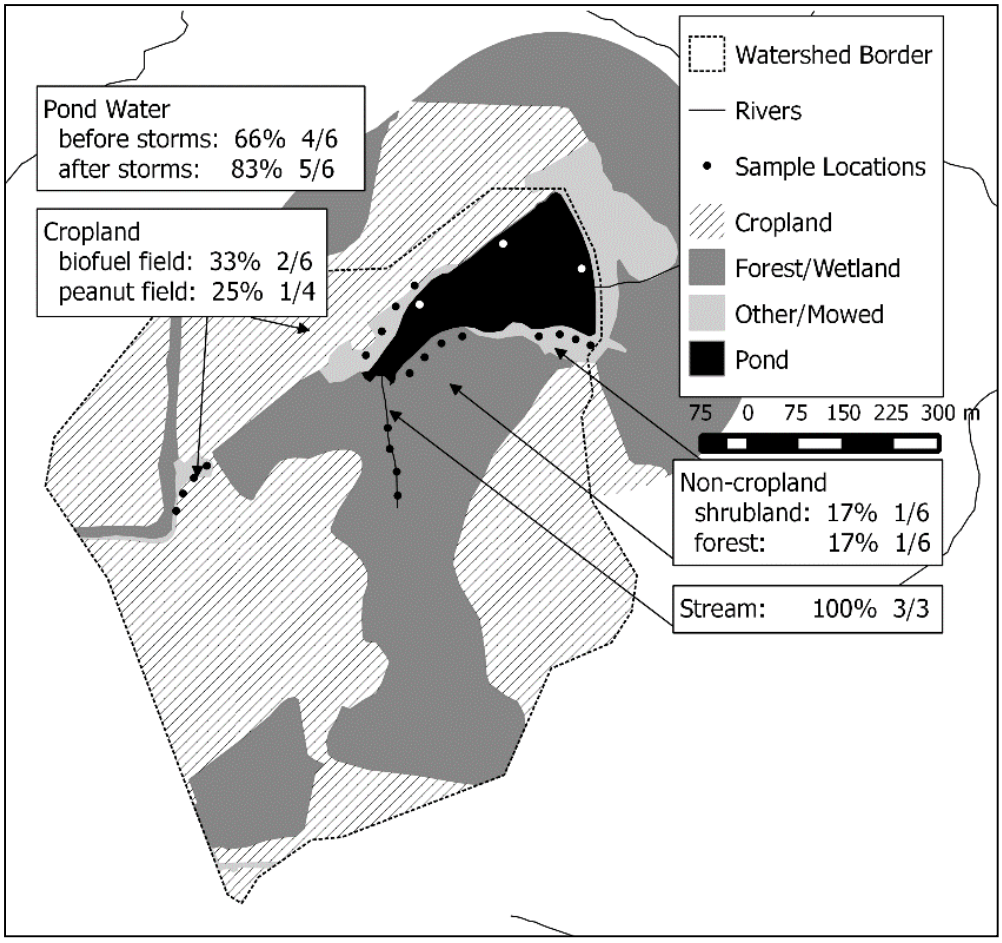


Figure 2.4
Log-transformed *Salmonella* concentrations in individual samples. Note that the data were natural log transformed after adding 0.0274 (half the lower limit of detection) to samples without detectable *Salmonella*, and log 0.0274 was subtracted from all transformed values to shift the scale to a more readable minimum value of 0.

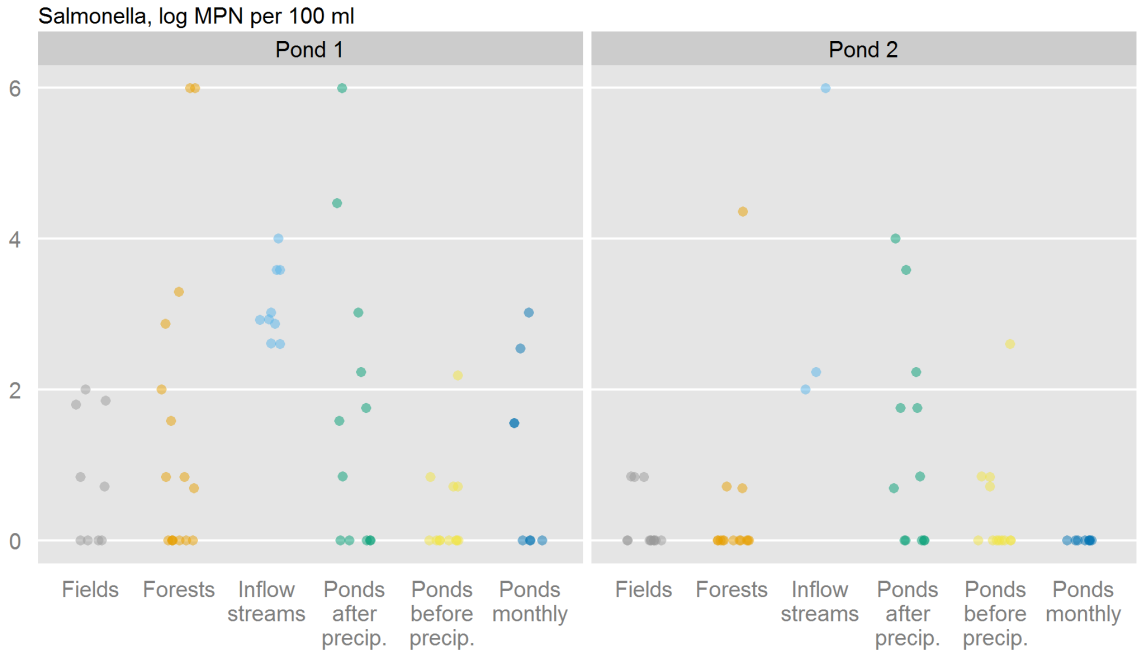


Figure 2.5
Log-transformed *E. coli* concentrations in individual samples.

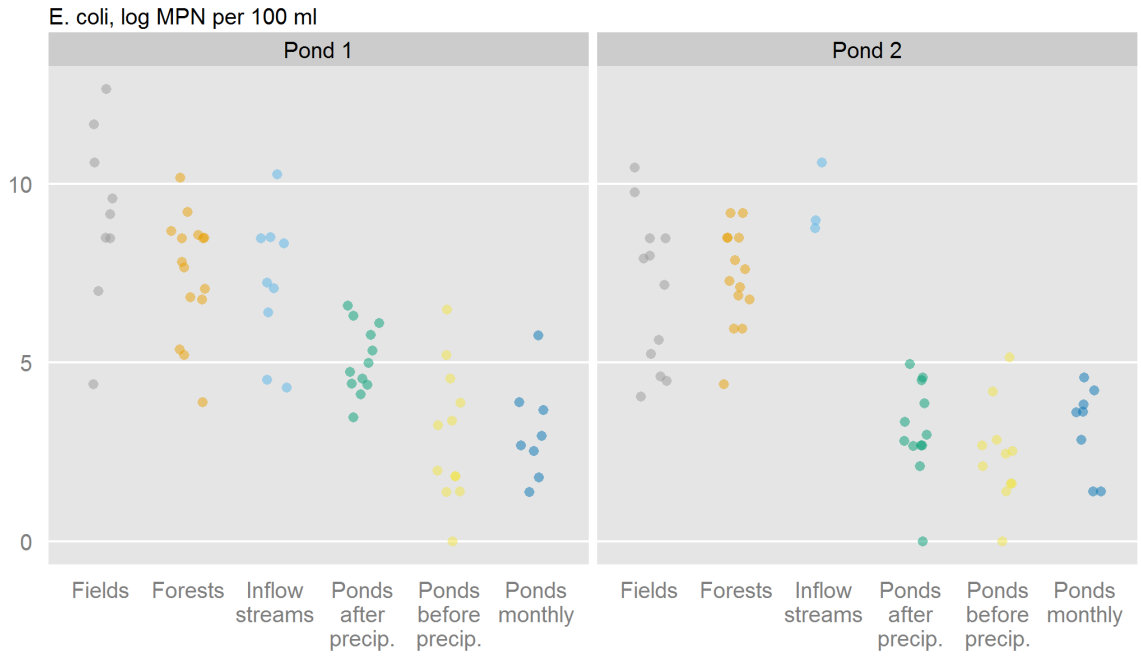


Figure 2.6
Log-transformed total solids in individual samples.

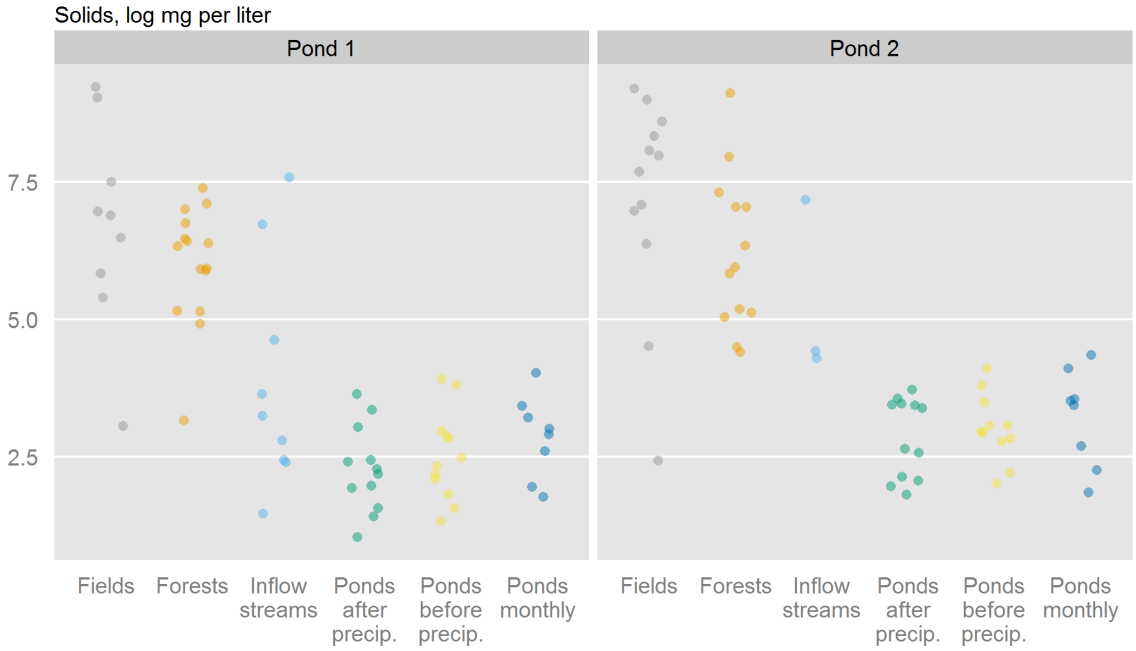


Figure 2.7
Model-estimated *Salmonella* concentrations by sample type. The data were transformed as in Figure 2.4. See results section for actual *Salmonella* concentrations. Statistically significant differences between sample types are indicated by non-overlapping 95% confidence intervals.

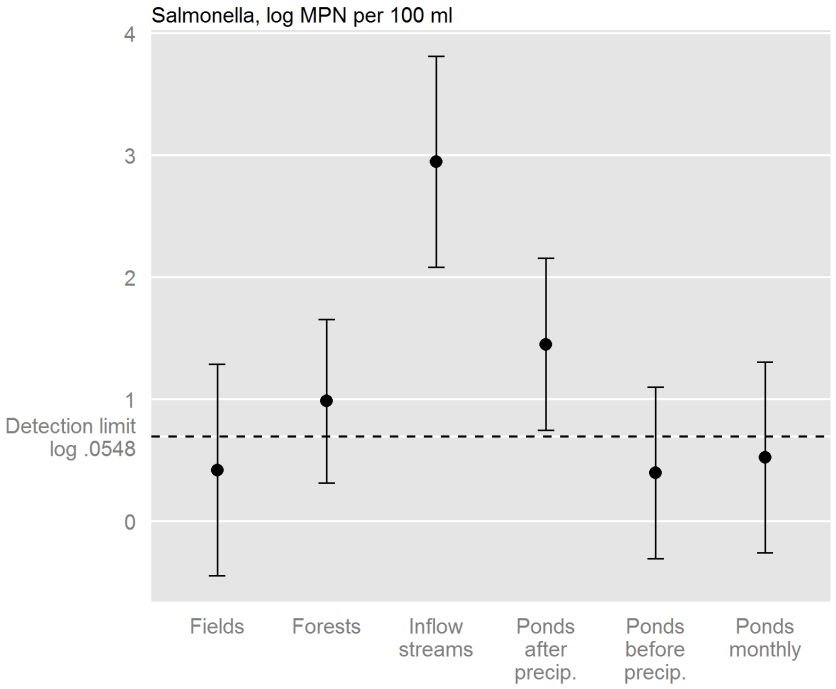


Figure 2.8
Model-estimated *E. coli* concentrations by sample type. The data were log-transformed; see results section for actual *E. coli* concentrations. Statistically significant differences between sample types are indicated by non-overlapping 95% confidence intervals.

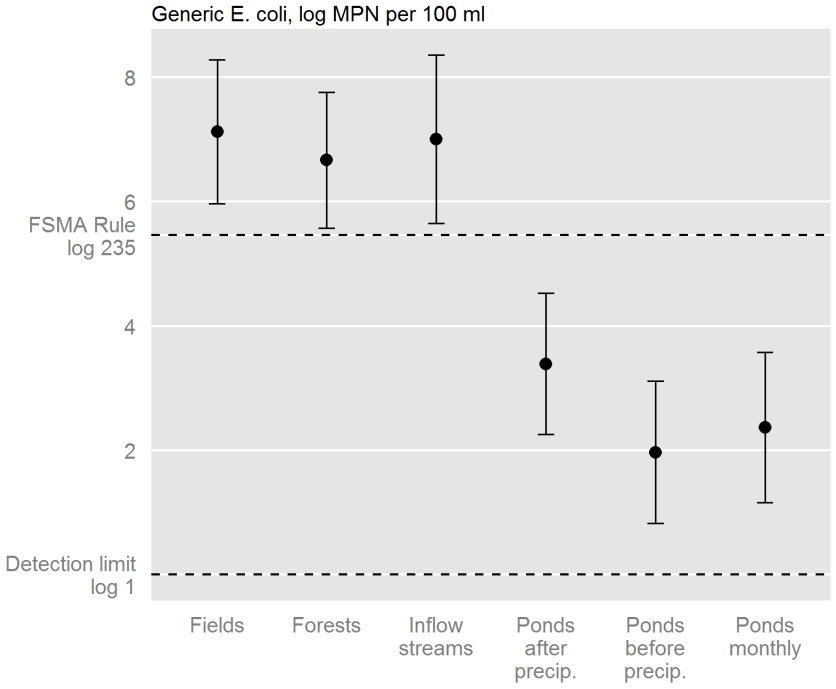


Figure 2.9
Model-estimated total solids by sample type. Statistically significant differences between sample types are indicated by non-overlapping 95% confidence intervals.

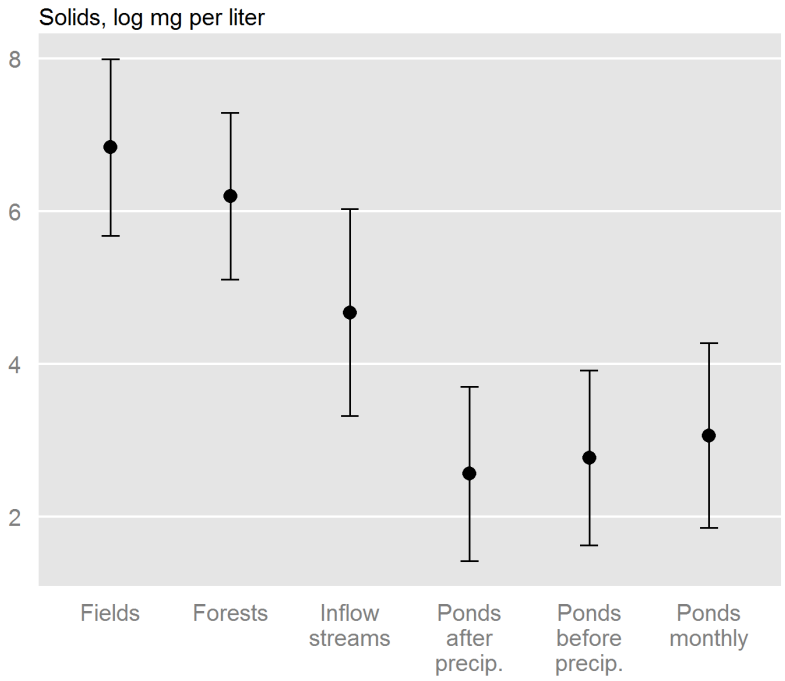


Figure 2.10
Salmonella concentrations in individual samples by date and sample type. Note that the data have been natural log transformed as in Figure 2.4.

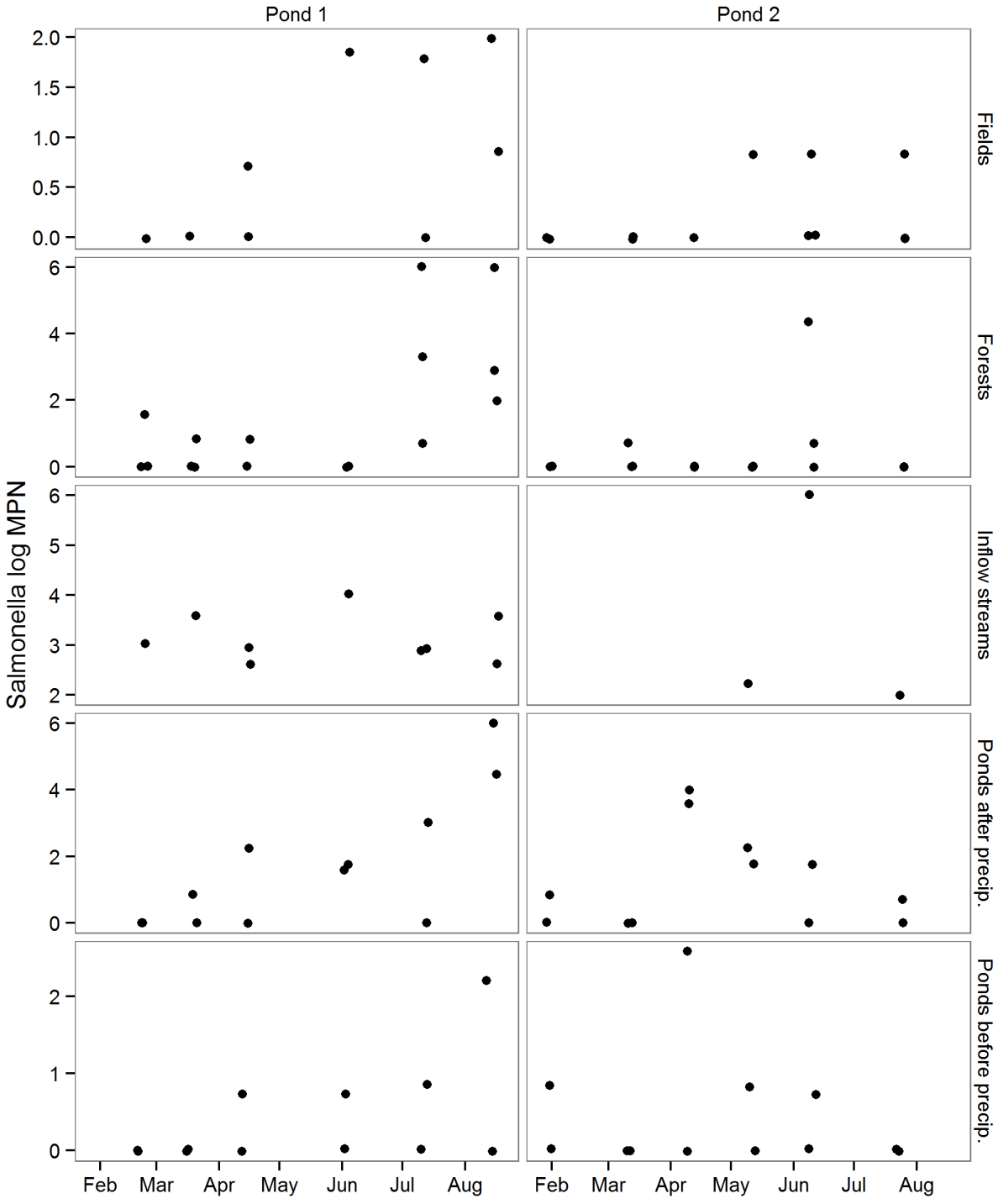


Figure 2.11

Concentrations of *Salmonella*, *E. coli*, and total solids vs. total rainfall within 48 hours prior to sample collection. Shown with Pearson's r values.

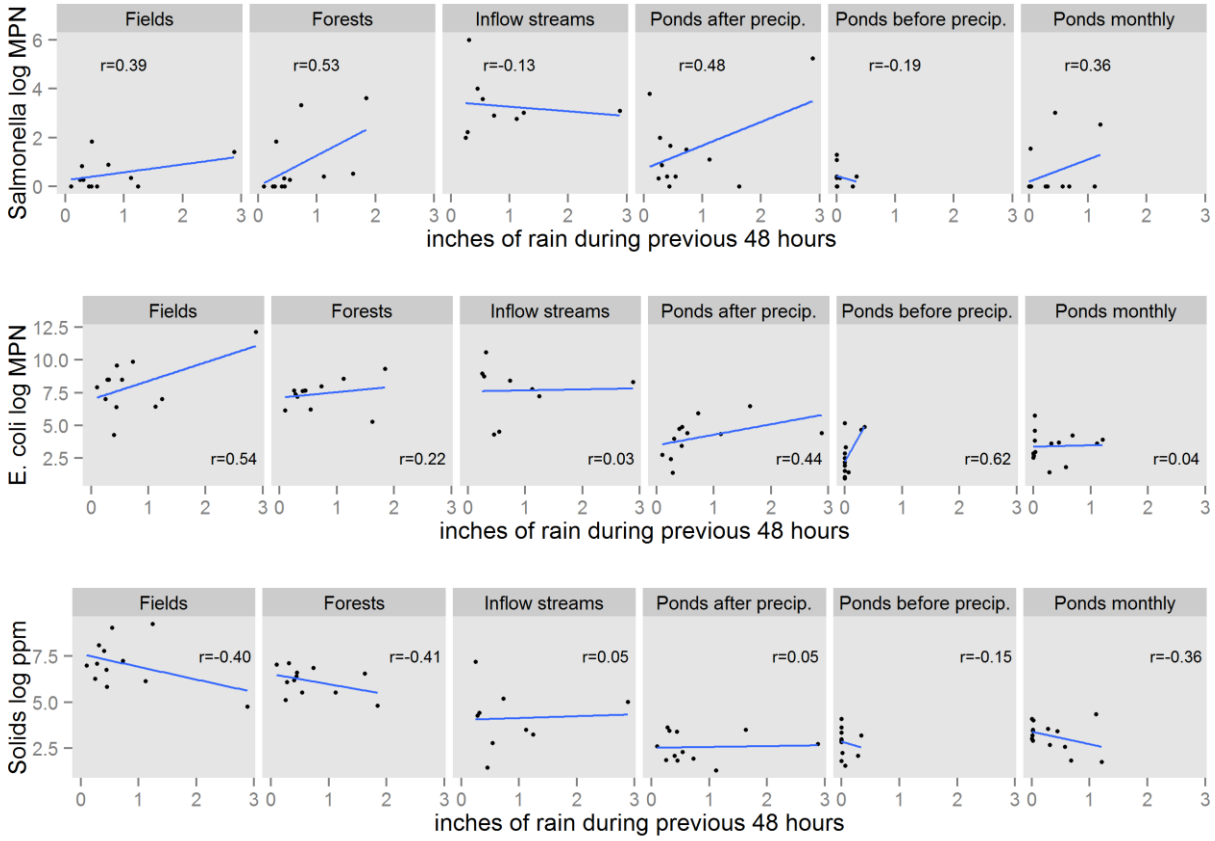


Figure 2.12

Concentrations of *Salmonella*, *E. coli*, and total solids vs. total rainfall within seven days prior to sample collection. Shown with Pearson's r values.

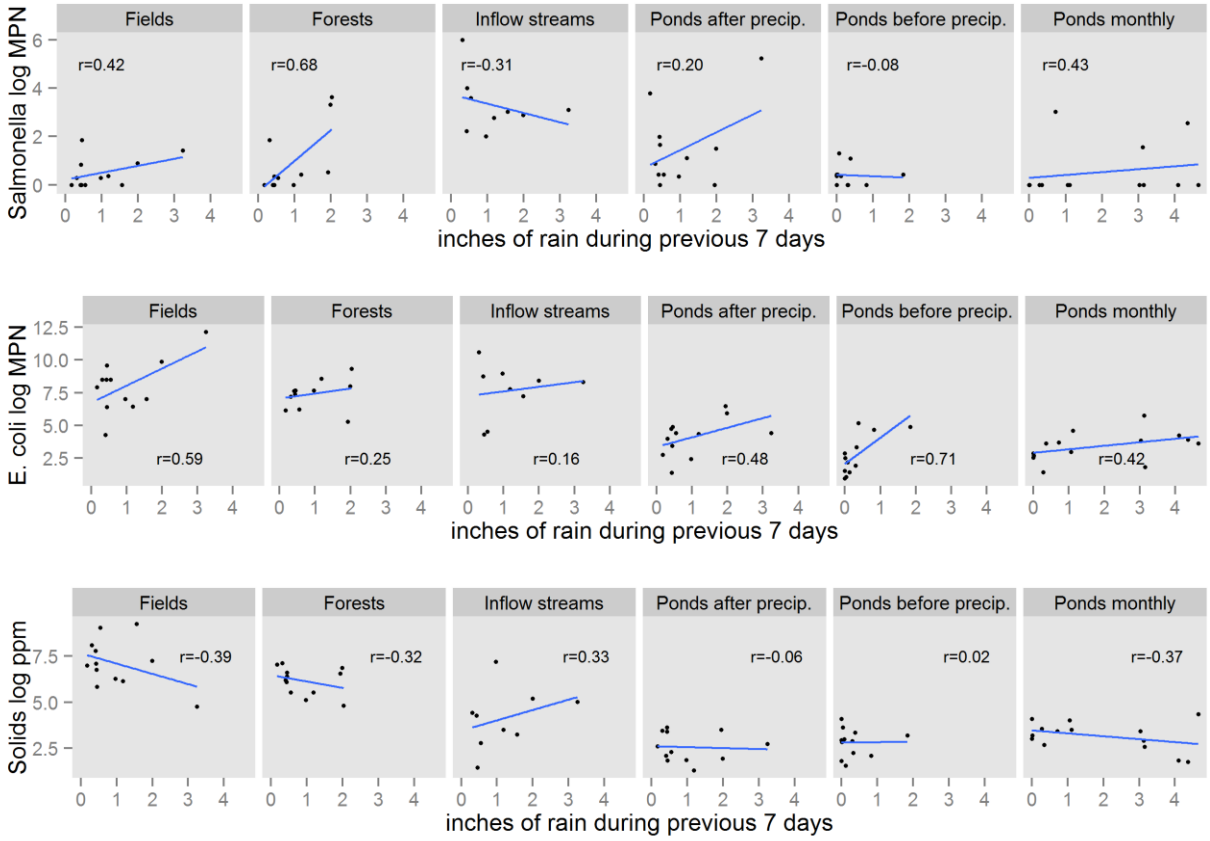
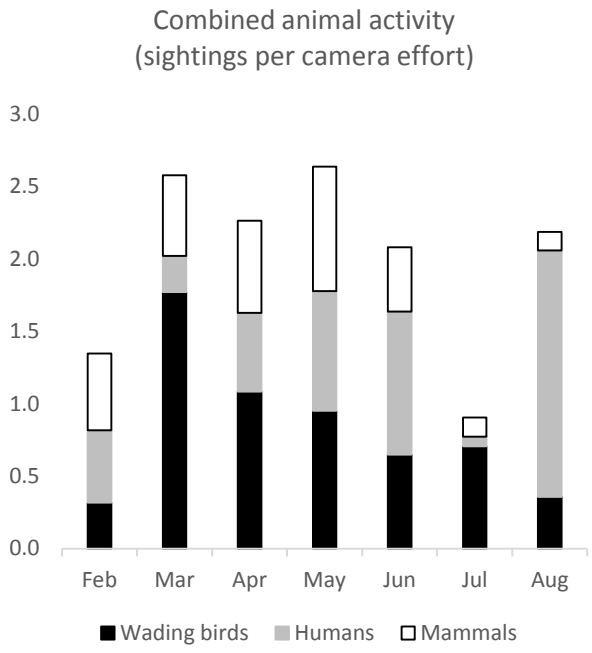
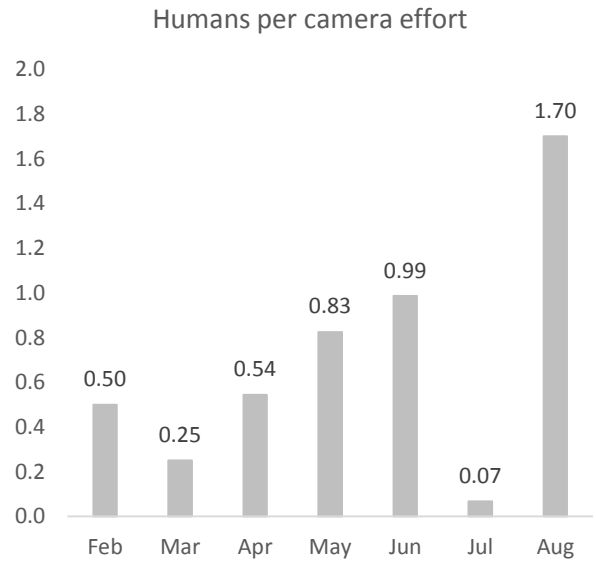


Figure 2.13
Animal activity by month. Values are for both Ponds 1 and 2 combined.

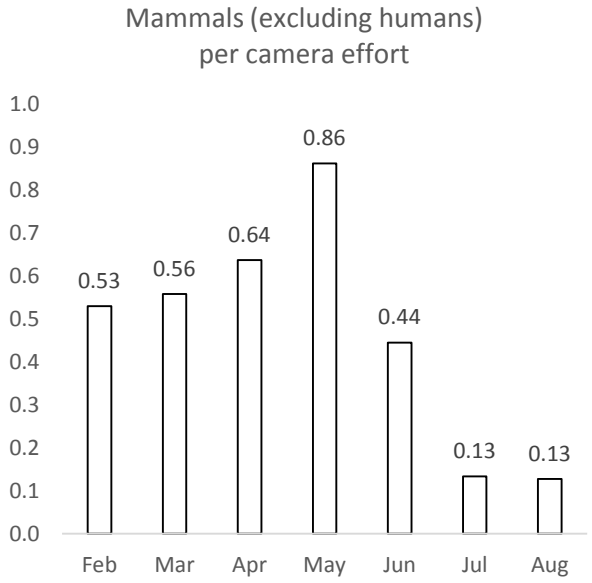
a)



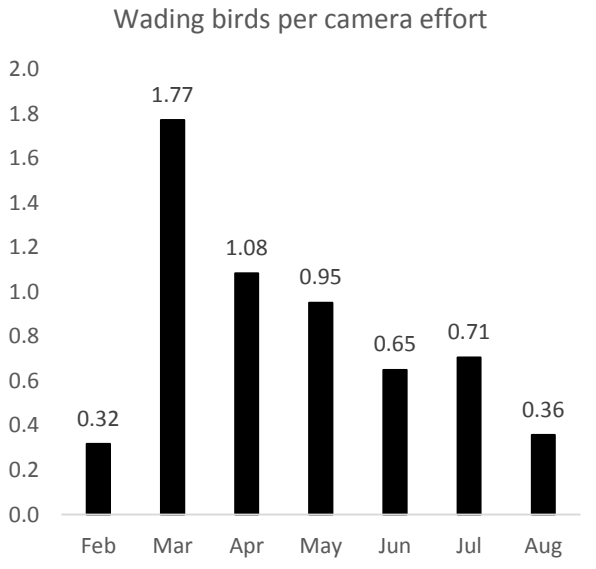
b)



c)



d)



CHAPTER 3

IMPACTS OF LAND COVER ON *SALMONELLA* CONCENTRATIONS IN FARM IRRIGATION PONDS IN RURAL SOUTH GEORGIA, USA[1]

[1] Harris, C.S., K. Levy, and G. Vellidis. To be submitted to *Applied and Environmental Microbiology*.

Abstract

Storm-driven surface runoff from land surfaces transports pathogens into waterways, where the presence of human pathogens is a growing concern. To examine the effects of different types of land cover on *Salmonella* concentrations in farm irrigation water, the landscapes surrounding ten irrigation ponds on commercial produce farms across rural southern Georgia, USA, were compared with the concentrations and prevalence of *Salmonella* in samples collected from the ponds monthly for one year. The proportions of different types of land cover (cropland, forest/wetland, developed, mowed, roads, pasture, and open water) were calculated for several different spatial extents surrounding each pond, including each pond's immediate watershed, a 250 m zone extending outward from the pond edges, a 250 m zone within the watershed only, and a 30.5 m zone buffering the pond edges. Simple linear regression was used to evaluate the relationships between developed, mowed, and forest/wetland land cover proportions and *Salmonella* prevalence or concentrations. Higher proportions of developed land cover (defined as residential or commercial buildings and adjacent maintained yards) across every spatial extent were significantly ($p < 0.05$) associated with higher *Salmonella* concentrations in irrigation ponds. Proportions of mowed land cover (primarily vegetated buffers) or forest/wetland land cover were not significantly associated with *Salmonella* prevalence or concentrations in irrigation ponds, except the proportion of mowed land cover within a 30.5 m zone buffering the pond edges which was significantly associated with lower *Salmonella* concentrations in ponds. Although only a small number of ponds were examined in this study, the results point to developed areas and associated human activities as possible important sources of *Salmonella* even in rural agricultural landscapes.

Introduction

One of the proposed requirements under the U.S. Food and Drug Administration's Food Safety Modernization Act (FSMA), the first major reform of U.S. food safety laws in over 70 years, is that many growers of fresh produce will be required to frequently test irrigation water for *Escherichia coli* or other fecal indicator bacteria (39, 100). This reflects a long-standing concern that fecal pollution affects waterways and by extension potentially our food supply. An estimated one in six people become ill from foodborne illnesses each year in the U.S., and nearly half these illnesses are likely acquired from raw or processed produce (1, 3). It is still unclear what the most important sources or pathways causing produce contamination may be, however. Irrigation water from surface freshwater sources is recognized as a potential reservoir of pathogens and a potential vehicle for the transmission of pathogens to plants and soil (72, 92), although only a few outbreaks of foodborne illness in the U.S. have been linked to contaminated irrigation water from surface freshwater sources (81–85). More than 60 million acres of cropland in the U.S. are irrigated by surface freshwater, requiring approximately 75 billion gallons of water withdrawals per day and highlighting the potential importance of water quality in food production (96).

Salmonella is the most common bacterial cause of foodborne illness in the U.S. (1). Outbreaks of *Salmonella* from tomatoes in 2002 and 2005 were linked to contaminated irrigation water in Virginia (81–85). Some *Salmonella* strains causing disease in humans have been shown to stick to plant surfaces and resist disinfectants, and to colonize seeds, sprouted seeds, leaves, and fruits (152–154). This makes contaminated irrigation water a concern in fruit/vegetable production, although *Salmonella* contamination may also occur during produce packing, processing, or distribution (155–157). *Salmonella* is commonly present in surface freshwater, and may enter waterways from a variety of sources and pathways. Studies from several agricultural regions of the U.S. have reported detectable *Salmonella* levels in 6% to 100% of surface water samples, although comparisons between studies are complicated due to

differences in sample volumes and analysis methods (48, 71, 74–77). Humans, domestic animals, livestock, and wildlife are all capable of carrying and spreading *Salmonella* (23, 156). Sewer system leakage, septic tanks, fecal deposition by domestic animals and wildlife, livestock, and manure use are all possible sources of *Salmonella* (47). Direct fecal deposition, surface runoff during storms, or subsurface flows through soils all may transport *Salmonella* to waterways (47, 94, 105), where it capable of surviving for many years in bottom sediments (19).

In this study, we sought to identify landscape-level land cover factors affecting *Salmonella* in farm ponds, and the spatial scales at which these factors matter. A few other studies have identified correlations between land cover and *Salmonella* concentrations or presence in waterways, but rarely specifically for irrigation ponds and never with fine-resolution land cover data (75, 86). Some potential *Salmonella* risks, such direct fecal deposition by wild animals into ponds, might be related to landscape characteristics such as habitat availability but not transported by storm runoff or subsurface flows through watersheds, so we evaluated land cover characteristics both at the watershed level and within various proximities to irrigation ponds where water samples were collected. We measured *Salmonella* in monthly pond water samples collected from irrigation ponds across southern Georgia, and used bivariate analysis to evaluate relationships between land cover and *Salmonella* in these ponds.

Materials and Methods

The Southeastern Plains and Southern Coastal Plain

The ten vegetable farm irrigation ponds in this study were located in the Southeastern Plains and Southern Coastal Plain ecoregions in inland southern Georgia, USA. These ecoregions span parts of the southeastern United States from Louisiana to Maryland, with a mild humid subtropical climate and a long growing season usually allowing two crops per year. Major crops include corn, soybeans, cotton, peanuts, onions, and a wide variety of fruits and vegetables. Land cover in this area consists of an uneven mosaic of cropland, pasture, successional pine-hardwood woodlands, large pine plantations, and woody

wetlands. The topography is mainly smooth and rolling plains, with lower relief and mostly flat plains toward the coast. The numerous streams in the region are generally slow-moving with sandy substrates. Larger rivers, wetlands, and increasingly wet soils are found toward the coast (109). Relatively few major lakes are present but many small man-made reservoirs and natural ponds are present along streams. An estimated 275,000 small (<2,000 ha) reservoirs and ponds exist across the state of Alabama, and a similar density is present in southern Georgia (98). Many of these ponds are used to collect and store surface freshwater for crop irrigation.

All ten irrigation ponds in this study were located on commercial mixed-produce farms within the Suwannee and Ochlockonee river basins, both of which drain into the Gulf of Mexico. Within the Suwannee and Ochlockonee river basins, ponds were located across seven subbasins (Figure 3.1). During periods of irrigation, some ponds were supplemented with groundwater.

Salmonella sample collection and analysis

Sampling of each pond was conducted once per month between January and December 2011, not necessarily on the same days at all ponds. In January and February, one water and one sediment sample were collected per pond. In March through July, two water samples and one sediment sample were collected per pond. In August through December, two water samples and two sediment samples were collected per pond. Water samples were collected near the irrigation pipe intake at the water's surface, and from a depth of about 0.5 m below the water's surface when an additional water sample was collected. Sediment samples were collected from the benthos below the irrigation pipe intake, and from the pond perimeter just below the water's surface when a second sediment sample was collected. Sediment samples were collected with a Wildco Mighty dredge grab sampler (Ben Meadows Co., Janesville, WI). Samples were collected with the aid of a boat, because many of the ponds' irrigation intake pipes were located a few meters from the shoreline. Samples were collected with sterile techniques and kept in dark coolers with ice during transport back to the laboratory.

Salmonella in water and sediment samples was identified using the same three-replicate, three-step (500, 100, and 10 ml for water; 100, 10, and 1 g for sediment) *Most Probable Number* (MPN) procedure and PCR confirmation previously described in detail in Chapter 2, with enrichment in non-selective broth followed by selective broth and plating on selective agars.

Land cover classification

The immediate watersheds surrounding each pond were determined using BASINS 4.1, a publicly available watershed mapping tool provided by the U.S. Environmental Protection Agency (available at water.epa.gov/scitech/datait/models/basins). Watersheds were calculated based on publicly available elevation data from the USGS National Elevation Dataset (available at nationalmap.gov). The watershed boundaries encompassed all of the land draining into each pond and did not include downstream areas. Watershed boundaries were examined for accuracy; areas in question were visited and watersheds were adjusted according to visual observations. Watershed size and pond size were calculated using tools available in QGIS 2.0.1, a free and open source mapping software (available at qgis.org).

Land cover within each watershed and within a 250 m radius surrounding each pond were visually classified and hand-digitized in QGIS 2.0.1 based on 2011 orthoimagery publicly available from the National Aerial Imagery Program (NAIP, nationalmap.gov) (Figure 3.2a-j). Publicly available land cover datasets including Georgia Land Use Trends (GLUT, narsal.uga.edu/glut) and the National Land Cover Dataset (NLCD, nationalmap.gov) did not classify land at high enough resolutions for the smaller spatial scales involved in this study, but these data layers were used as guides for areas of orthoimagery that were difficult to visually classify. Land cover was hand-digitized down to a very fine spatial resolution (more precise and more accurate) than GLUT or NLCD, although this resolution was not specifically defined.

Seven classifications were specified for land cover: cropland, forest or wetland, developed areas, mowed areas, open water, pasture, and public roads. Cropland consisted of agricultural row crops as well

as edible tree or shrub crops such as pecans or blueberries. Forests and wetlands consisted of natural, minimally disturbed areas as well as planted or managed pines. Mowed areas consisted of herbaceous non-wetland cover, including grass on roadsides, areas beneath power line right-of-ways, and numerous mowed field borders and filter strips associated with cropland. Developed areas consisted of rural homes and commercial buildings, as well as any maintained yard spaces immediately adjacent to those buildings. Roads included paved and unpaved public roads. Unpaved roads serving only agricultural fields were considered part of cropland. A few pastures (areas with recent livestock presence) were identified, but in general it was not possible to definitively identify active pastures, especially forested areas potentially used for grazing. Hay fields without visible cow paths were counted as cropland.

Land cover calculations

Digitized land cover layers were "clipped" to several different spatial extents using QGIS 2.0.1. These spatial extents of interest were the full watershed of each pond as calculated from BASINS 4.1, land within a 250 m radius of each pond edge, land within a 250 m radius of each pond edge only within each pond's watershed, and land within a 30.5 m radius of each pond edge. Ponds themselves were not considered part of the land cover layer. Geoprocessing tools in QGIS were used to outline the relevant spatial extents, and a geometry calculator in QGIS was used to determine the areas of land cover parcels digitized from orthoimagery within each spatial extent of interest. To normalize land cover data, we converted the areas of each land cover type present within each spatial extent of interest to the proportion (percent) of total area (Tables 3.2-3.3).

Statistical analysis

All data was natural log-transformed prior to analysis to achieve a normal distribution (evaluated using Q-Q plots). A value of 0.0274, half the lower limit of detection for the MPN method used, was assigned to samples with no detectable *Salmonella.* Mean *Salmonella* concentration and prevalence were used as indices of *Salmonella* at each pond. Mean *Salmonella* concentration for each pond was calculated

by first determining the average concentration of water samples collected in each month, then averaging all twelve months together (n=12). *Salmonella* prevalence at each pond was defined as the number of positive samples out of the total number of sediment + water samples (n=39) collected at each pond. Bivariate linear regression analyses were performed for *Salmonella* indices and three land cover types of interest: forest/wetland, mowed areas, and developed areas within each of the four spatial extents. Pearson's r was used to compare bivariate correlations, and *p*-values were determined to evaluate the significance of these correlations. A positive or negative Pearson's r-value indicates a positive or negative relationship, respectively, between variables. An r-value closer to 1 or -1 indicates a steeper slope and closer fit of a linear regression line, while a value closer to 0 indicates a flat regression line or no relationship. Corrections for multiple comparisons were not used, although it should be noted that a total of 34 tests were performed. A paired t-test was used to compare samples collected during summer months with samples collected during winter months at each pond (n=10).

Ponds A and B were eliminated from statistical analyses related to land cover and watershed/pond size. These two ponds had unusual topological and hydrological conditions compared to ponds C through J. Pond A had an extremely large watershed encompassing a large recreational lake draining into two subbasins, and the surrounding topography appeared more similar to the karst topography of Florida with frequent circular sinkholes than to the typical topography of the Tifton-Vidalia Upland of southern Georgia which is dominated by dense dendritic networks of streams. Pond B was isolated from its watershed by raised berms constructed to increase its water holding capacity. It was recharged primarily by pumped groundwater rather than surface runoff.

Results

Salmonella

Thirty-nine sediment and water samples were collected from each of the ten ponds during 2011. All of the ponds had detectable levels of *Salmonella* during at least one month. For convenience, the

letters A through J were assigned to the ponds to reflect decreasing *Salmonella* prevalence. The percentage of *Salmonella*-positive samples collected from each pond during 2011 ranged from 10 ± 9% (standard error of proportion) to 51 ± 14% (Table 3.1, Figure 3.3a). Summer samples, collected from April through September, had significantly higher *Salmonella* prevalence than samples collected in winter months from the same ponds (p = .002) (Figure 3.3c and 3.3e).

The mean concentrations of *Salmonella* in water samples ranged from 0.028 MPN per 100 ml to 0.144 MPN per 100 ml, with large standard deviations of at least 1 MPN per 100 ml back-calculated from log standard deviations (Table 3.1, Figure 3.3b). For comparison, the lower limit of detection for the MPN method used was 0 .0548 MPN per 100 ml. Summer samples collected from April through September had significantly higher *Salmonella* concentrations than samples collected in winter months from the same ponds (p = .010) (Figure 3.3d and 3.3f).

Land cover analysis

No land cover types were highly cross-correlated with one another within any spatial extent (r did not exceed 0.8). The dominant proportion of land cover at all spatial scales was cropland or forest/wetland, except at pond B which had an extremely small watershed comprised almost entirely of mowed berms (Tables 3.2-3.3). Cropland surrounding the rest of the ponds was primarily fruit/vegetables or row crops (corn, cotton, or soybeans). Cropland characteristics of note included a large plantation of blueberry shrubs near Pond J, and a large plantation of pecan trees near Pond C. No developed areas were located at pond B or I within the pond watersheds or within 250 m of the pond edges. The watersheds of ponds D and G contained recognizable cattle pastures, although it was not known how intensively or how recently these pastures had been used.

Mean *Salmonella* concentrations in pond water consistently and significantly ($p < 0.05$) increased with the percentage of developed areas in the pond watersheds or closer to the ponds (Table 3.5, Figure 3.5a-b), although this relationship appeared to be particularly influenced by Pond C. Inconsistent and

non-significant relationships were observed for mean *Salmonella* concentrations and mowed or forest/wetland land cover (Figures 3.6a-b and 3.7a-b). None of the bivariate correlations between *Salmonella* prevalence and various land cover proportions were statistically significant, although some weak trends appeared (Table 3.4). *Salmonella* prevalence seemed to consistently increase with the percentage of developed areas in the pond watersheds or closer to the ponds. Inconsistent relationships were observed for *Salmonella* prevalence and mowed or forest/wetland land cover.

Watershed and pond size

Watershed sizes for the ten ponds ranged from 0.01 km^2 to 9.21 km^2. Pond sizes ranged from 5,799 m^2 to 79,255 m^2 (Table 3.1). Neither watershed size nor pond size were significantly correlated with either *Salmonella* prevalence or concentrations (Tables 3.4-3.5, Figures 3.4a-b).

Discussion

This study measured *Salmonella* prevalence and concentrations in irrigation ponds surrounded by the range of landscapes typically found in agricultural areas in the southeastern coastal plain of the U.S., and used detailed land cover data to identify relationships between *Salmonella* and land uses within various proximities to the ponds. Examining landscape metrics at various spatial scales has been identified as a useful strategy for landscape analyses (158). Other studies have examined land use characteristics in relation to *Salmonella* or other pathogens in waterways, but have used coarser-resolution land cover data and have not focused specifically on irrigation ponds (75, 86, 159). Other studies have focused on single types of land use (145), or comparisons between watersheds with different dominant land cover types (60, 91).

Mean *Salmonella* concentrations were higher in ponds surrounded by greater proportions of developed land cover. This relationship was consistently significant at the watershed scale and at closer proximities to the ponds, although it appeared to be influenced by Pond C. Sources of *Salmonella* in areas of residential and commercial development may include leaky septic tanks, domestic animals, small

numbers of backyard livestock, and wildlife adapted to living in close proximity to humans (57, 60, 146, 160). Many households in this region use septic tanks, which have been linked to improved *Salmonella* survival rates in soils (71, 146). A study of waterways in central California noted higher concentrations of *Salmonella* in streams within primarily urban watersheds compared to forested watersheds (86), and within urban stream reaches compared to forested stream reaches (145). Other studies in Canada and Australia have also noted high concentrations of *Salmonella* in urban waterways (60, 144). Although the present study was conducted exclusively in rural watersheds rather than urban watersheds, these findings suggest that some of the same human-related factors leading to increased *Salmonella* concentrations in urban watersheds may be present to a lesser degree, but still important, in rural watersheds.

Proportions of forest/wetland or mowed land cover did not show clear or consistent relationships with *Salmonella* concentrations or prevalence. Wildlife is a potential source of *Salmonella* in these types of areas. A study in California identified *Salmonella* occasionally in birds, coyotes, deer, pigs, and skunks, all of which are present in south Georgia as well, and other studies have identified *Salmonella* in reptiles and small rodents (59, 161, 162). Forest/wetland and mowed areas in southeastern agricultural landscapes can harbor significant populations of reptiles and small rodents (author, personal observation), but these vegetated zones also serve to trap and filter waterborne contaminants before they reach irrigation ponds (163). To some extent, vegetated areas may be a source as well as a sink for *Salmonella.*

Higher proportions of mowed land cover within 30.5 m of ponds appeared to be related to lower *Salmonella* concentrations. Maintaining vegetated areas around ponds and waterways has long been viewed as a good agricultural practice for soil conservation and improved water quality (107, 108). The National Resources Conservation Service recommends using riparian herbaceous buffers at least 10 m wide between cropland and water sources (108, 164). Mowed areas, when managed correctly to ensure

evenly distributed storm flow percolation, can reduce the levels of nutrients, pesticides, sediments, and pathogens in runoff entering downstream waterways (165–167). However, some growers of fresh produce have recently converted vegetated buffers to bare ground due to concerns about wildlife presence (65, 67). In an unpublished study of rodent populations near ponds involved in the present study, mice and rats were trapped frequently in forested areas adjacent to ponds as well as in mowed vegetated buffers and very narrow (<1 m) sparsely vegetated strips between ponds and dirt roads.

In the present study, *Salmonella* concentrations in individual water samples never exceeded 1 MPN per 100 ml. A quantitative microbial risk assessment using *Salmonella* surrogates in irrigation water in Arizona suggested that *Salmonella* levels above 2.5 MPN per 100 ml in irrigation water could cause illness in 1 per 10,000 consumers, under the worst-case scenario that produce is harvested, processed, packaged, and consumed only one day after the last irrigation event (73). It remains unclear whether *Salmonella* is in fact a likely contributor, rather than a potential or rare contributor, to foodborne illness through contaminated irrigation water.

Identifying the specific factors associated with increased *Salmonella* concentrations in proximity to developed areas in agricultural watersheds is an important area for future research. Many fruit and vegetable growers are well aware of the microbiological risks of having livestock upstream from irrigation water sources, especially after multiple outbreaks of highly pathogenic *E. coli* were reported from lettuce irrigated with water contaminated by cattle feces (81, 82), but it is unclear which human-related factors may be most relevant to the contamination of irrigation water. Future studies could move upstream to sample particular sources of *Salmonella* in developed environments, and use genetic sequencing to compare *Salmonella* strains from developed environments and irrigation water. Improving riparian buffer zones and storm water flow paths will also be important for reducing pathogen inputs to irrigation ponds and limiting the potential for contamination.

Tables

Table 3.1

Summary of *Salmonella* data by pond, with watershed and pond sizes.

Pond	Percent of samples (water and sediment) positive for *Salmonella*	Avg monthly *Salmonella* concentration (MPN per 100 ml)	Watershed area (km^2)	Pond area (m^2)
A	51%	0.144	9.21	18490
B	44%	0.074	0.01	5799
C	33%	0.073	0.20	10955
D	33%	0.050	0.88	21637
E	26%	0.035	0.23	46421
F	23%	0.037	2.75	79225
G	21%	0.050	3.27	35518
H	18%	0.044	0.22	16148
I	15%	0.035	0.92	38914
J	10%	0.028	0.66	46010

Table 3.2

Proportion of total land cover by type for each pond watershed, and for each pond watershed within 250 m of the pond edge. Dominant cover types for each pond and extent are shown in bold. Crop=cropland, Dev=developed, Mow=mowed herbaceous, Nat=forest and wetland, Other=open water, public roads, and identifiable pastureland.

	Watershed					Watershed within 250 m				
Pond	Crop	Dev	Mow	Nat	Other	Crop	Dev	Mow	Nat	Other
A	0.11	0.21	0.07	**0.36**	0.26	**0.71**	0.00	0.06	0.17	0.06
B	0.00	0.00	**0.91**	0.09	0.00	0.00	0.00	**0.91**	0.09	0.00
C	**0.36**	0.23	0.13	0.28	0.00	**0.35**	0.25	0.15	0.25	0.00
D	**0.44**	0.02	0.06	0.37	0.11	**0.53**	0.02	0.02	0.41	0.02
E	**0.77**	0.00	0.09	0.13	0.01	**0.70**	0.00	0.11	0.17	0.01
F	**0.60**	0.03	0.06	0.26	0.05	0.33	0.05	0.20	**0.40**	0.01
G	**0.45**	0.06	0.02	0.38	0.09	**0.45**	0.00	0.01	0.41	0.14
H	**0.83**	0.02	0.03	0.08	0.04	**0.76**	0.03	0.05	0.11	0.06
I	0.21	0.09	0.10	**0.58**	0.02	0.28	0.12	0.14	**0.46**	0.00
J	**0.63**	0.00	0.03	0.34	0.00	**0.49**	0.00	0.07	0.45	0.00

Table 3.3

Proportion of total land cover by type within 250 m and 30.5 m radii of each pond's outer edge. Dominant cover types for each pond and extent are shown in bold. Crop=cropland, Dev=developed, Mow=mowed herbaceous, Nat=forest and wetland, Other=open water, public roads, and identifiable pastureland.

	Within 250 m of pond					Within 30.5 m of pond				
Pond	Crop	Dev	Mow	Nat	Other	Crop	Dev	Mow	Nat	Other
A	**0.49**	0.00	0.03	0.45	0.03	**1.00**	0.00	0.00	0.00	0.00
B	**0.80**	0.00	0.04	0.15	0.01	0.41	0.00	**0.42**	0.17	0.00
C	0.29	0.18	0.21	**0.32**	0.00	0.00	0.25	0.32	**0.42**	0.00
D	**0.57**	0.01	0.02	0.34	0.06	0.07	0.00	0.00	**0.91**	0.02
E	**0.51**	0.02	0.11	0.35	0.01	0.22	0.00	0.29	**0.46**	0.03
F	**0.43**	0.03	0.13	0.40	0.01	0.01	0.00	**0.82**	0.16	0.01
G	0.33	0.02	0.04	**0.51**	0.10	0.00	0.00	0.05	**0.90**	0.05
H	**0.76**	0.03	0.02	0.15	0.04	0.39	0.00	0.18	**0.43**	0.00
I	0.29	0.07	0.10	**0.53**	0.01	0.00	0.16	**0.51**	0.33	0.00
J	0.37	0.00	0.09	**0.54**	0.00	0.22	0.00	**0.52**	0.26	0.00

Table 3.4

Land cover predictors vs. the proportion of *Salmonella*-positive samples collected at each pond, for ponds C through J only. Based on simple linear regression analysis. No correlations were statistically significant. Degrees of freedom for *F* tests were 1 (numerator) and 6 (denominator, number of ponds - 2) for each regression.

Simple linear regression analysis:
Land cover vs. *Salmonella*-positive sample **proportion** per pond

Predictors	Pearson's r	R^2	Regression slope	*F*	Significance (p)
Watershed area	-0.11	0.01	-0.01	0.07	0.80
Pond area	-0.34	0.11	0.00	0.77	0.41
Percent Land Cover					
Watershed					
Developed	0.44	0.19	0.46	1.41	0.28
Mowed	0.49	0.24	1.06	1.92	0.21
Forest/Wetland	-0.20	0.04	-0.10	0.24	0.64
Watershed within 250 m of ponds					
Developed	0.41	0.17	0.38	1.20	0.31
Mowed	0.09	0.01	0.11	0.05	0.83
Forest/Wetland	-0.25	0.06	-0.15	0.41	0.54
Within 250 m of ponds					
Developed	0.45	0.21	0.62	1.56	0.26
Mowed	0.26	0.07	0.33	0.43	0.54
Forest/Wetland	-0.45	0.21	-0.28	1.56	0.26
Within 30.5 m of ponds					
Developed	0.29	0.08	0.24	0.55	0.49
Mowed	-0.37	0.14	-0.11	0.95	0.37
Forest/Wetland	0.44	0.19	0.13	1.40	0.28

Table 3.5

Land cover predictors vs. the average monthly *Salmonella* concentrations of water samples collected at each pond, for ponds C through J only. Based on simple linear regression analysis. No correlations were statistically significant. Degrees of freedom for *F* tests were 1 (numerator) and 6 (denominator, number of ponds - 2) for each regression. Significant correlations ($p < 0.05$) are shown in bold.

Simple linear regression analysis:

Land cover vs. average monthly *Salmonella* concentration per pond

Predictors	Pearson's r	R^2	Regression slope	*F*	Significance (p)
Watershed area	-0.01	0.00	0.00	0.00	0.974
Pond area	-0.65	0.42	0.00	5.14	0.064
Percent Land Cover					
Watershed					
Developed	**0.75**	0.56	2.84	10.11	0.019
Mowed	0.34	0.12	2.65	1.20	0.315
Forest/Wetland	-0.09	0.01	-0.17	0.08	0.782
Watershed within 250 m of ponds					
Developed	**0.62**	0.39	2.09	7.01	0.038
Mowed	-0.09	0.01	-0.37	0.09	0.772
Forest/Wetland	-0.28	0.08	-0.61	1.11	0.333
Within 250 m of ponds					
Developed	**0.71**	0.51	3.50	14.52	0.009
Mowed	0.23	0.05	1.06	0.87	0.388
Forest/Wetland	-0.43	0.18	-0.95	3.57	0.108
Within 30.5 m of ponds					
Developed	**0.53**	0.28	1.58	6.69	0.041
Mowed	-0.50	0.25	-0.54	5.98	0.050
Forest/Wetland	0.48	0.23	0.51	5.69	0.054

Figures

Figure 3.1

Map of the study area. The ponds were located in the Upper Ochlockonee, Withlacoochee, Alapaha, Lower Ocmulgee, and Upper Suwannee subbasins (one pond each), the Lower Ochlockonee subbasin (two ponds), and the Little subbasin (three ponds).

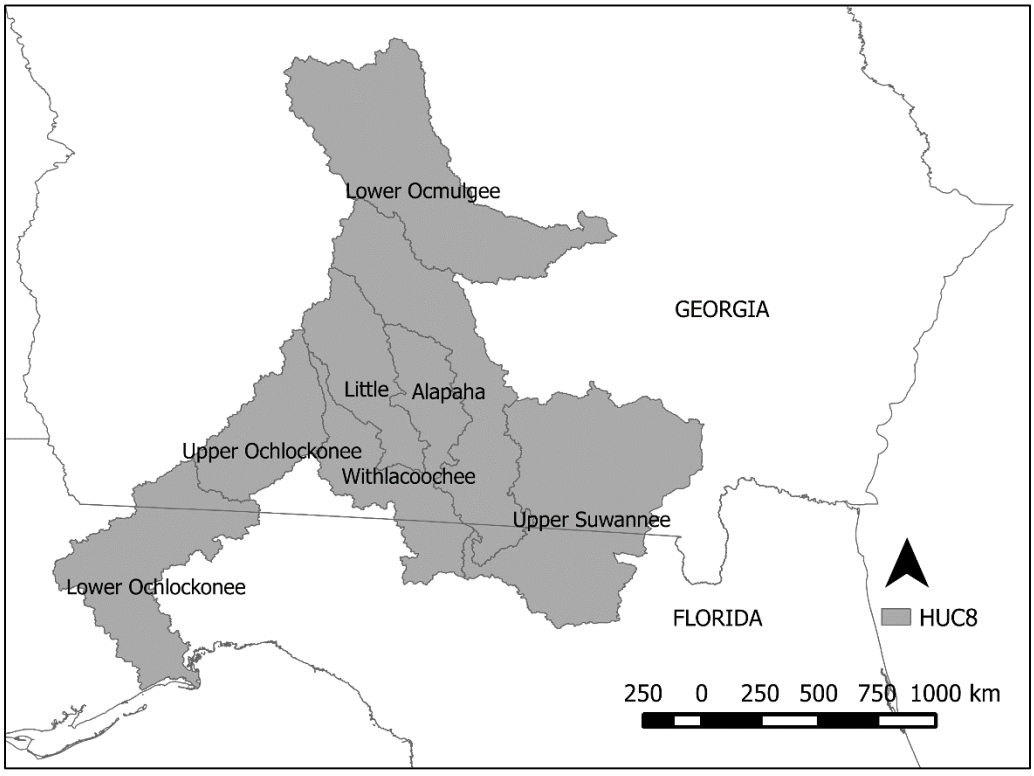
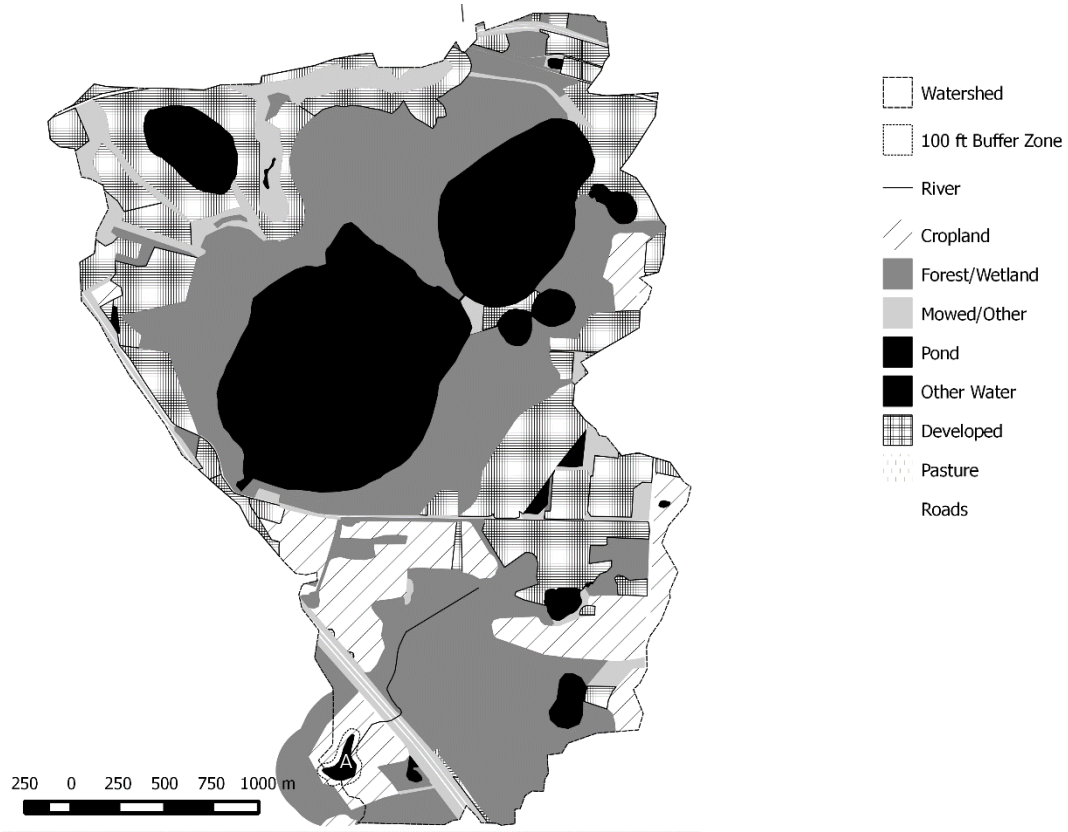


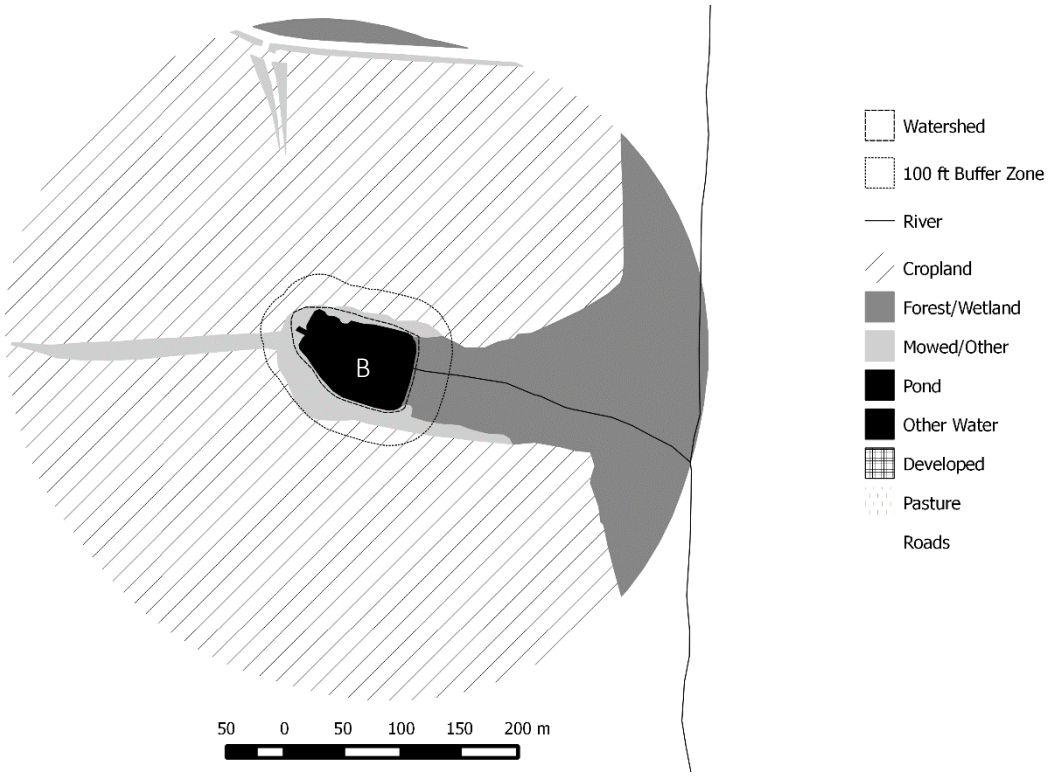
Figure 3.2a-j

Map of pond landscapes. Ponds A through J (highest proportion (A) to lowest proportion (J) of samples positive for *Salmonella*). Land cover is shown for the watershed extent and within 250 m of the pond.

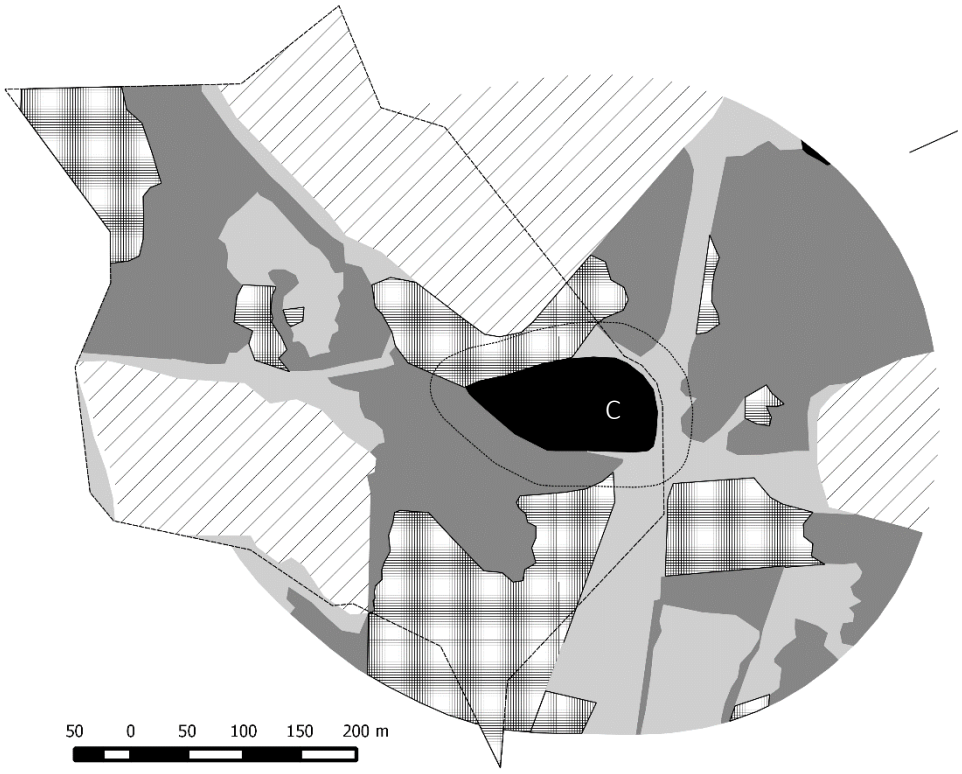
3.2a Pond A



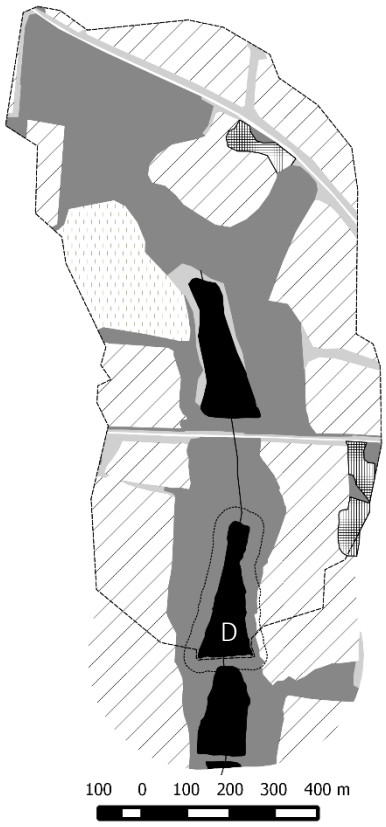
3.2b Pond B



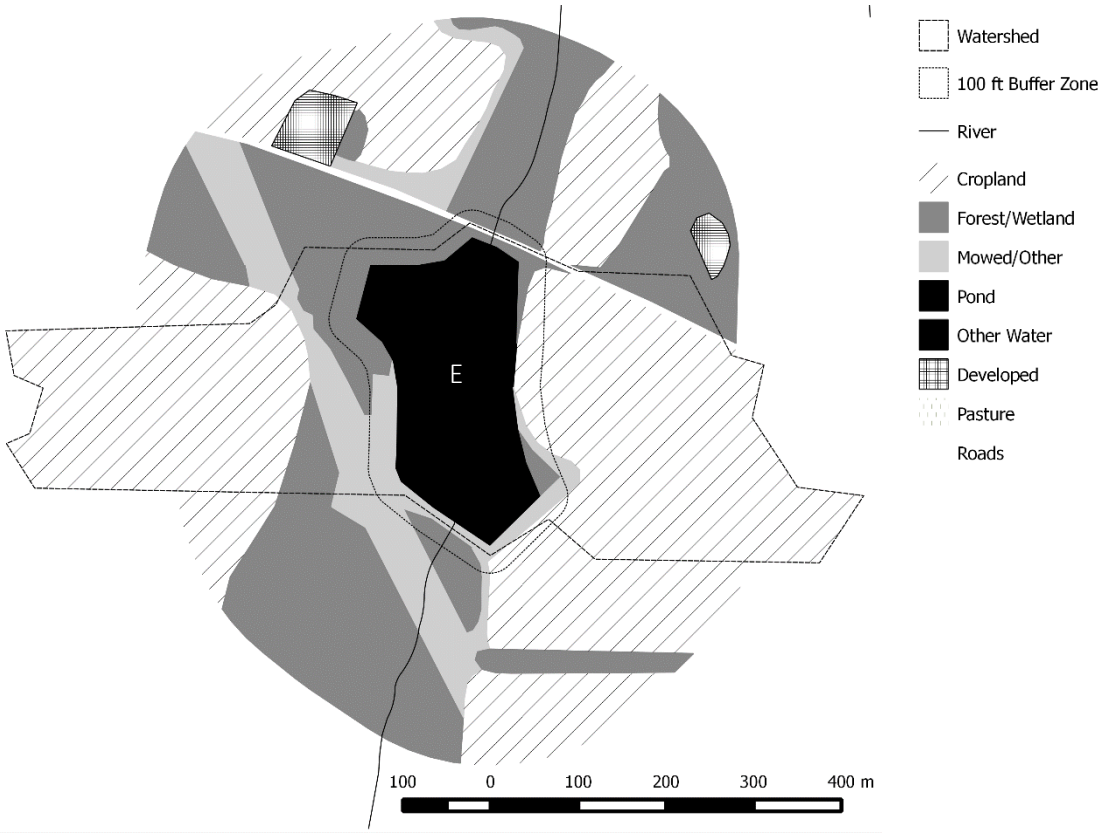
3.2c Pond C



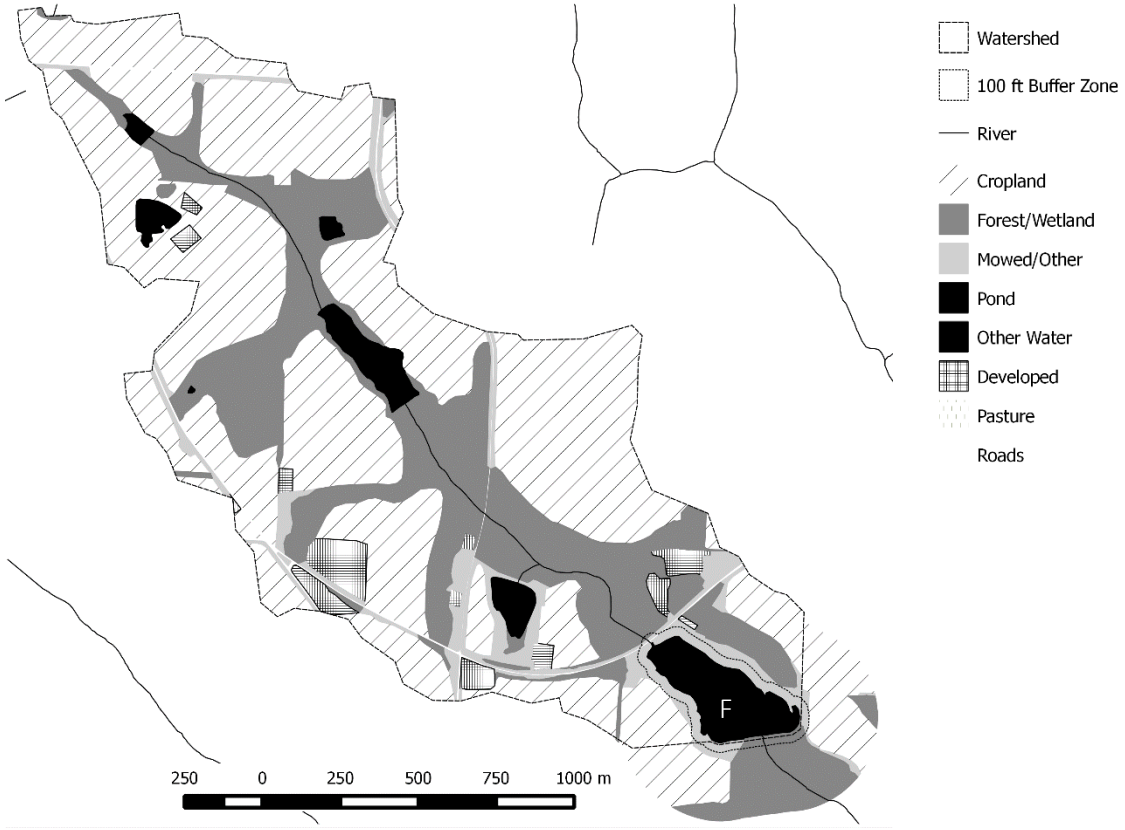
3.2d Pond D



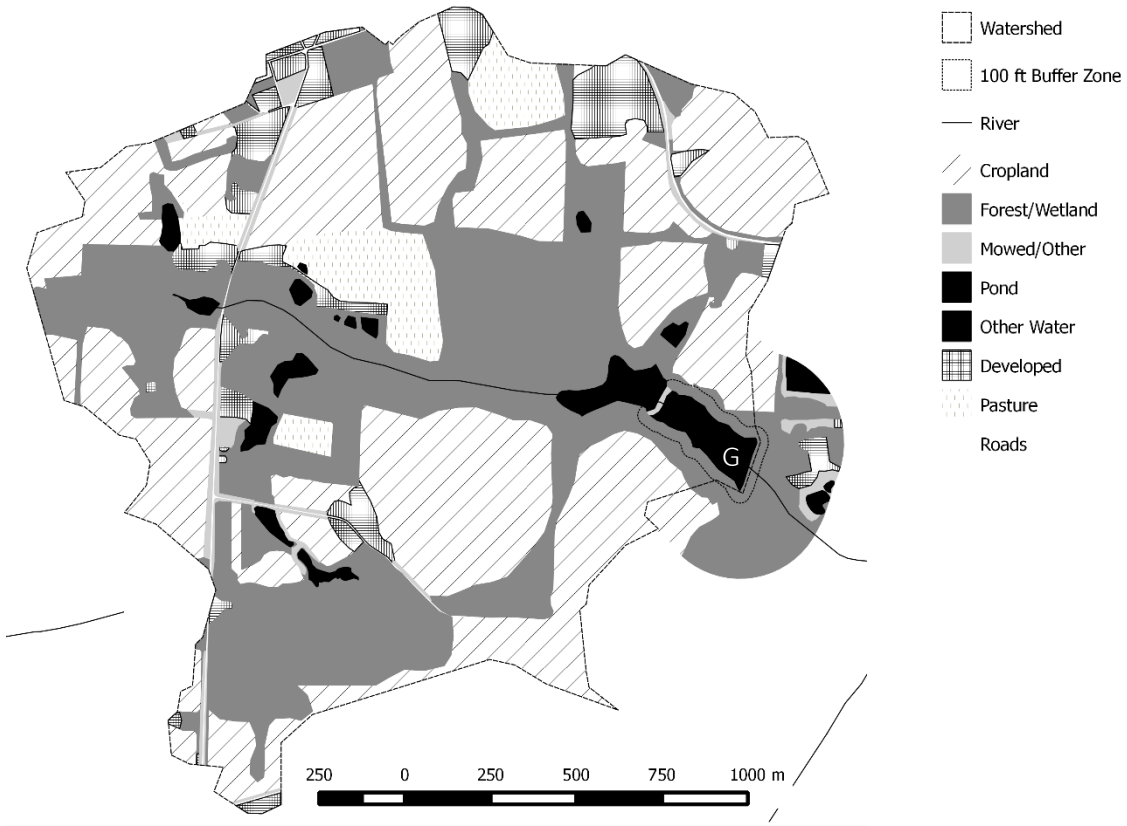
3.2e
Pond E



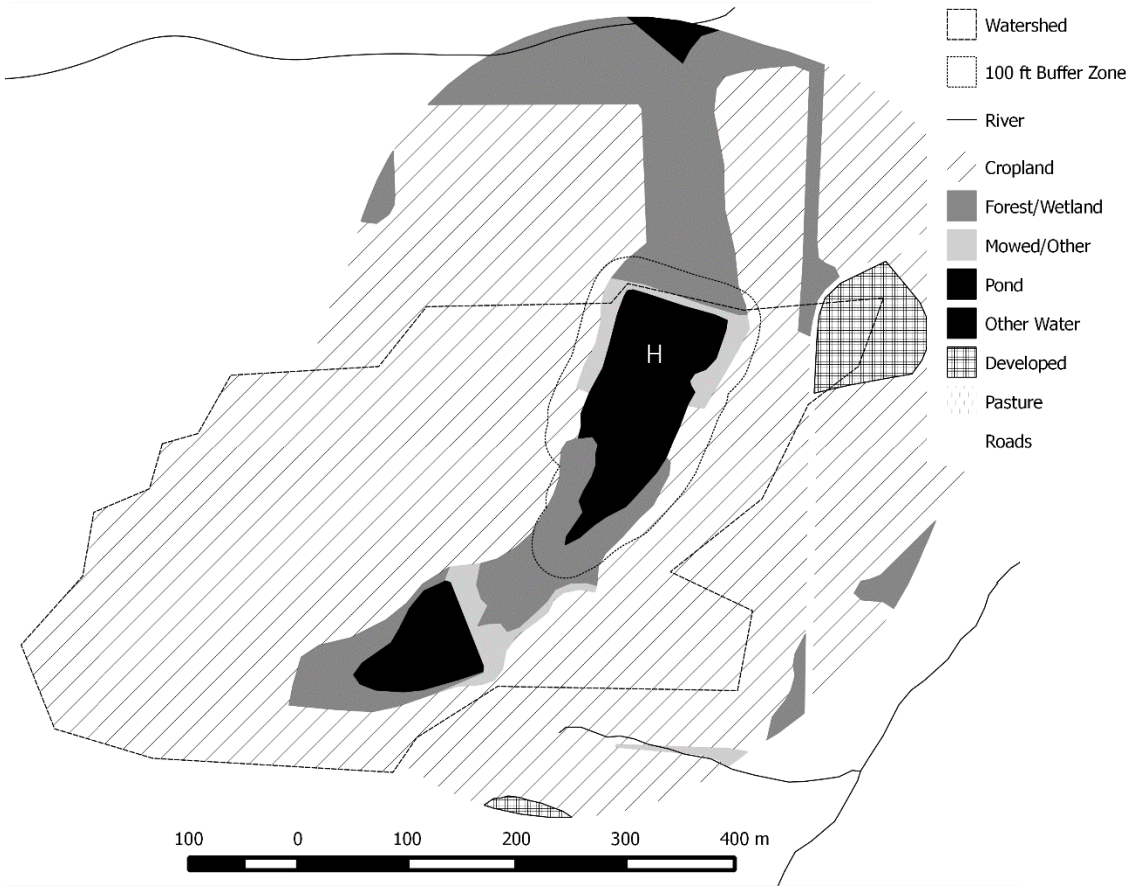
3.2f
Pond F



3.2g
Pond G



3.2h
Pond H



3.2i Pond I

3.2j Pond J

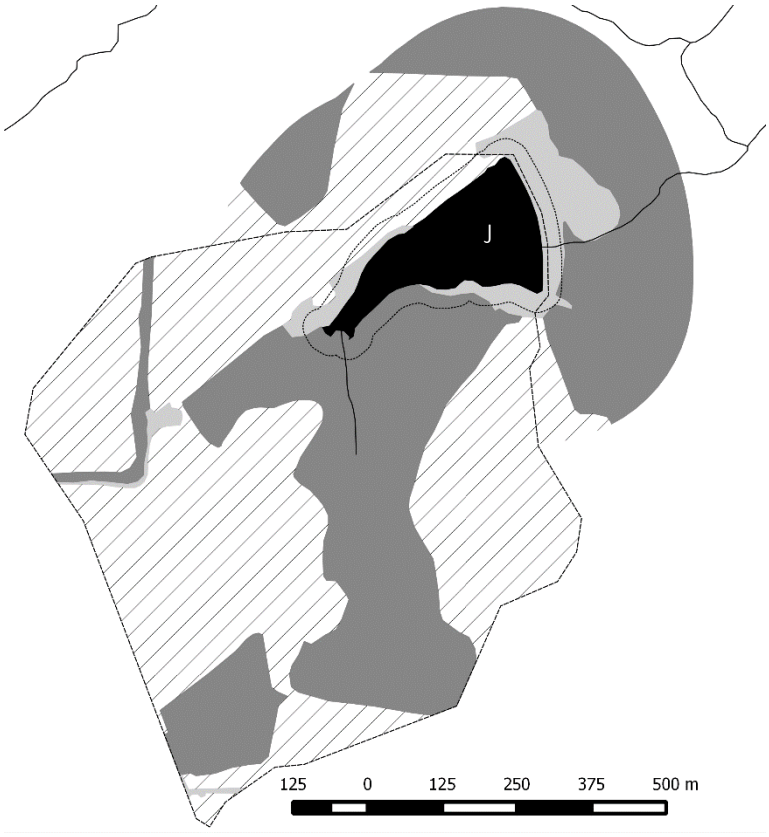
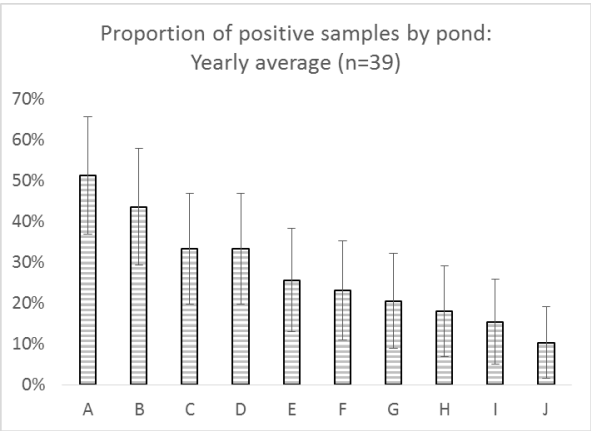


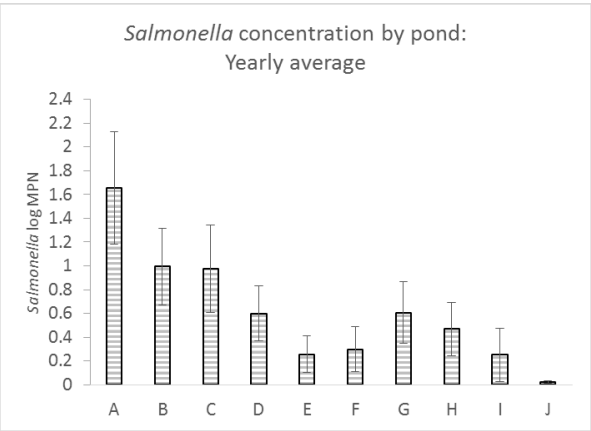
Figure 3.3a-f

Seasonal proportions of positive samples and concentrations of *Salmonella* by pond. Year-round, summer-only, and winter-only averages are shown. Proportions of positive samples are based on water and sediment samples (shown with the standard error of proportion). Concentrations are based on average log-adjusted water samples per month per pond (shown with standard error). The lower limit of detection on the adjusted log scale is 0.69 adj. log MPN per 100 ml.

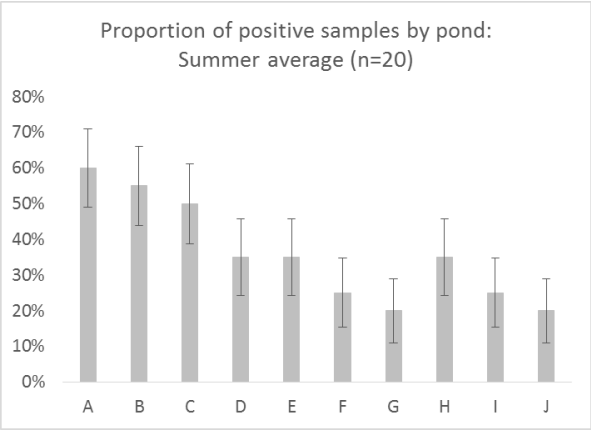
3.3a



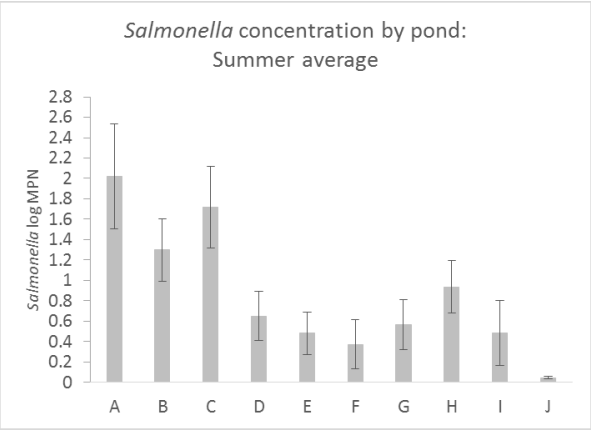
3.3b



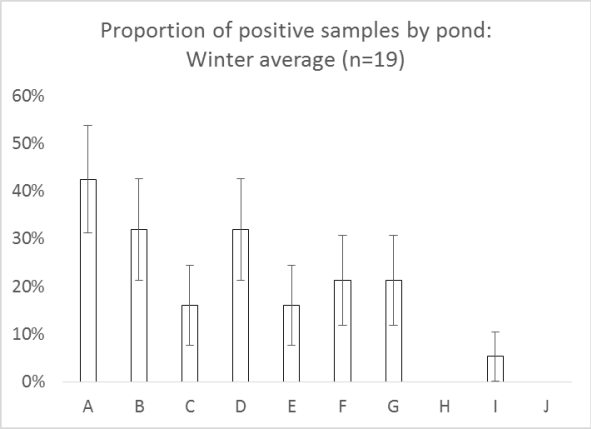
3.3c



3.3d



3.3e



3.3f

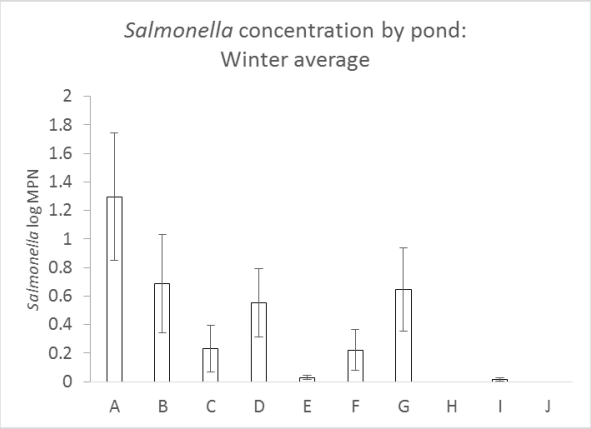
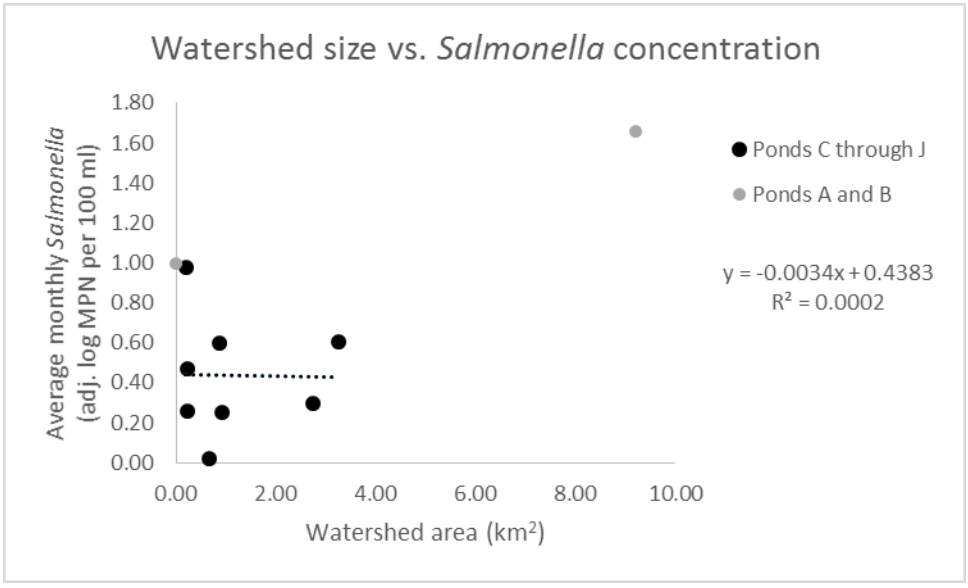


Figure 3.4a-b
Watershed and pond area vs. *Salmonella* concentrations. Trendlines are based on data from ponds C through J. Ponds A and B are shown for comparison.

3.4a



3.4b

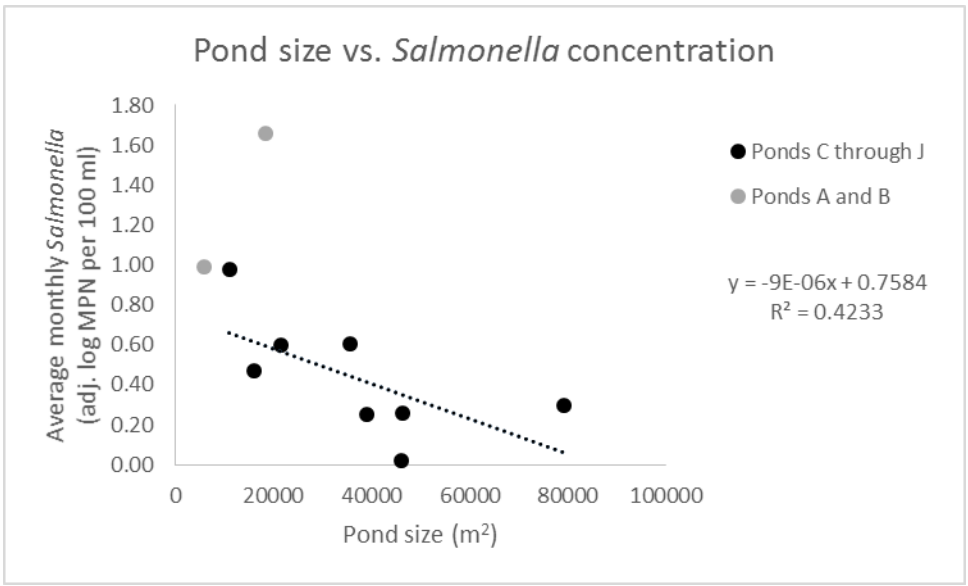
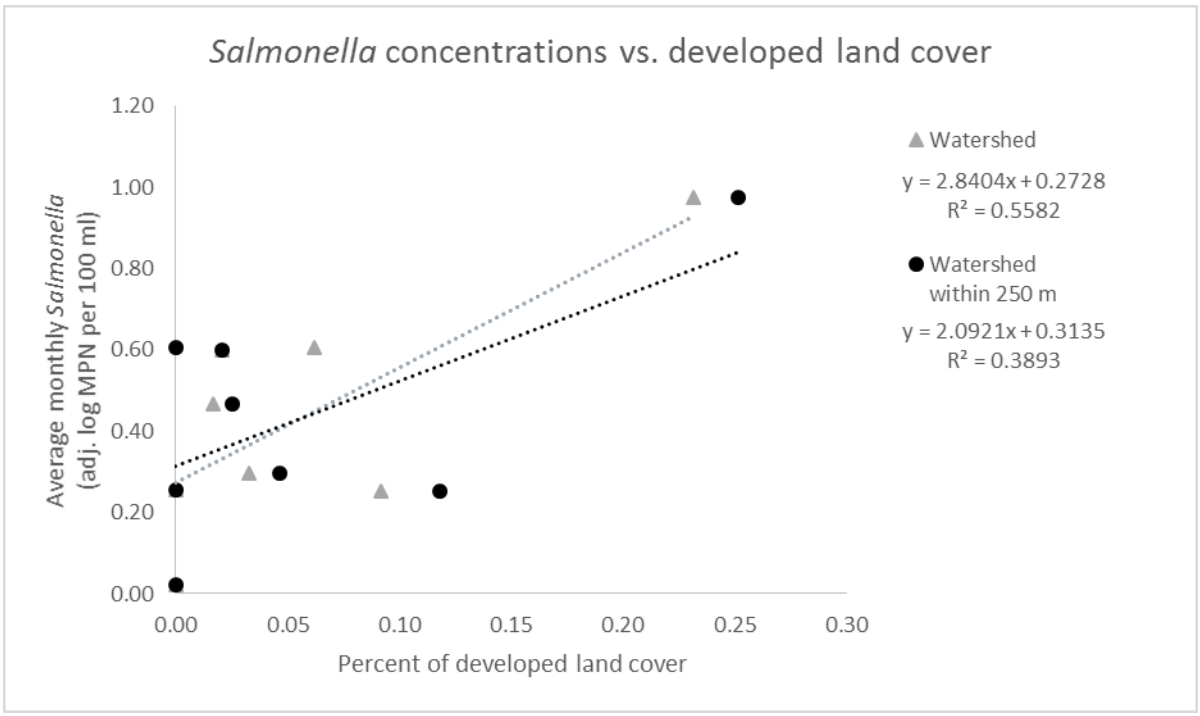


Figure 3.5a-b
Developed land cover vs. *Salmonella* concentrations. Shown for several spatial extents, ponds C through J only.

3.5a



3.5b

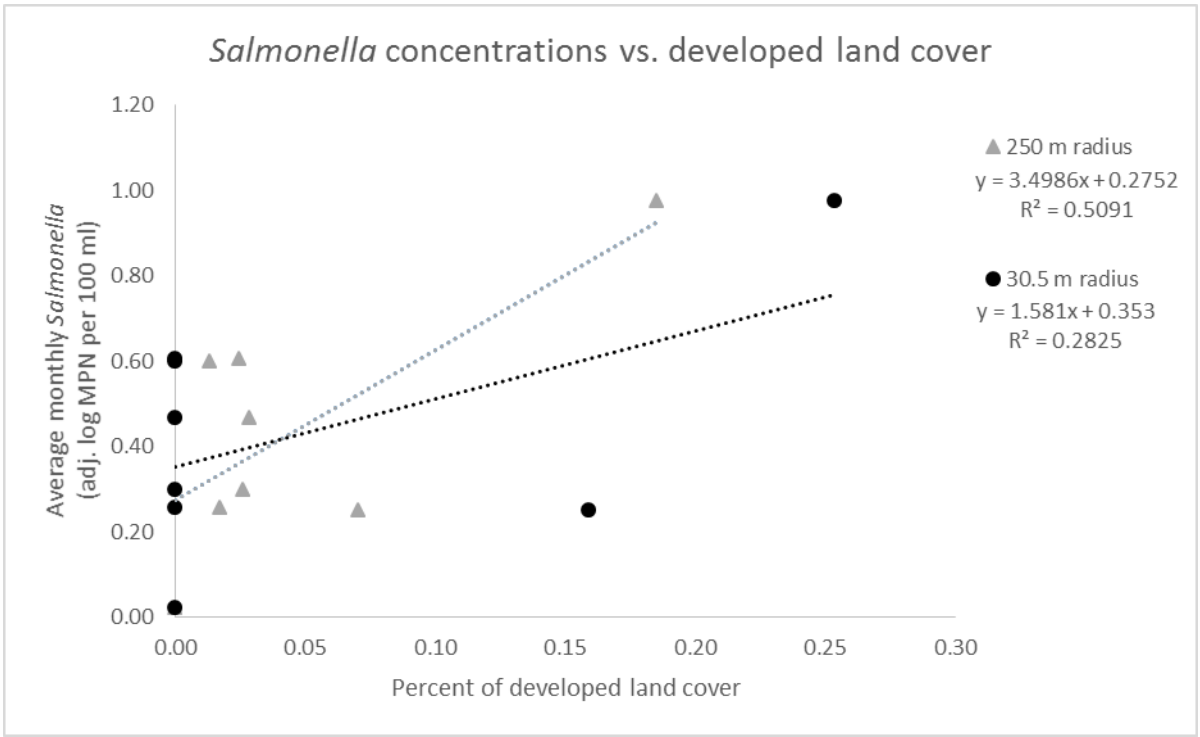
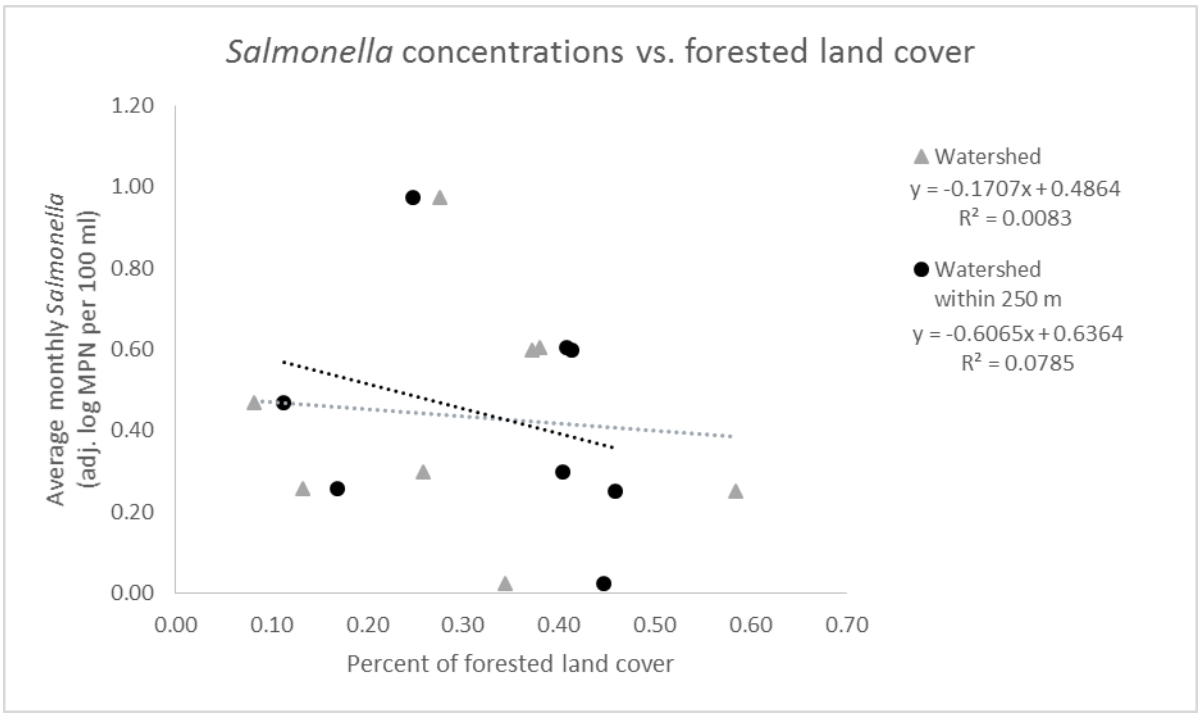


Figure 3.6a-b
Forested land cover vs. *Salmonella* concentrations. Shown for several spatial extents, ponds C through J only.

3.6a



3.6b

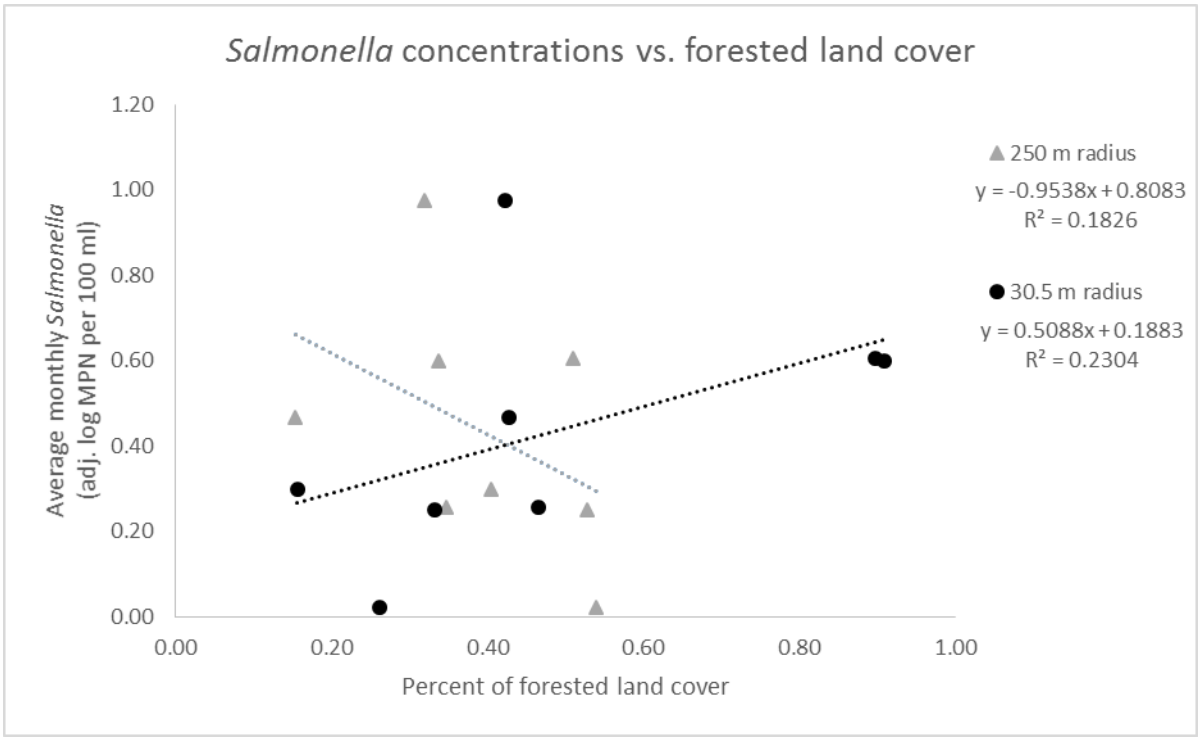
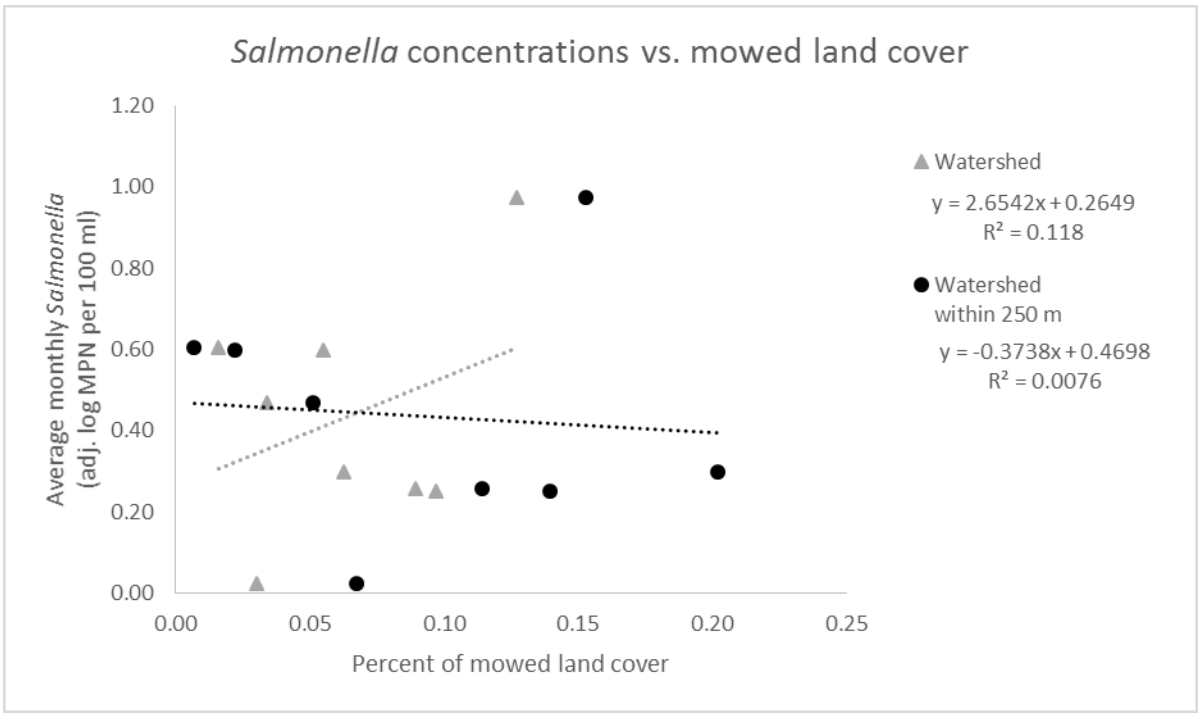
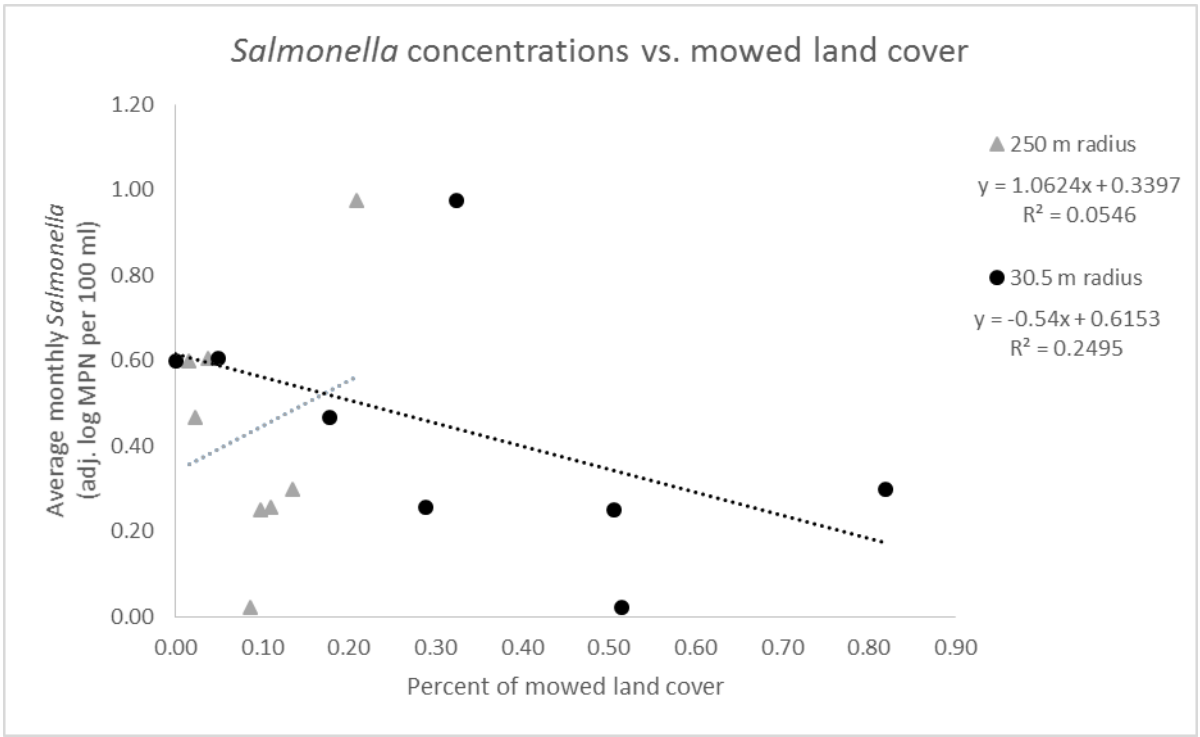


Figure 3.7a-b
Mowed land cover vs. *Salmonella* concentrations. Shown for several spatial extents, ponds C through J only.

3.7a



3.7b



CHAPTER 4

SUMMARY AND RECOMMENDATIONS

Salmonella is frequently found in agricultural irrigation ponds, although usually in low concentrations. *Salmonella* presence can be expected in irrigation ponds connected to natural waterways, transported to ponds by stream flow or storm-driven surface runoff. The most significant findings of our studies suggest that 1) storm flow in streams may have significantly higher *Salmonella* concentrations than storm-driven surface runoff from crop fields or forested areas, 2) *Escherichia coli* concentrations are not an adequate predictor of *Salmonella* concentrations in irrigation ponds, 3) irrigation ponds may regularly contain *Salmonella* serotypes commonly implicated in human illness, and 4) *Salmonella* concentrations appear to be higher in irrigations ponds surrounded by higher proportions of developed (residential and commercial) land cover, also suggesting the importance of human-related sources of *Salmonella* in agricultural irrigation ponds even in rural watersheds.

Important questions now facing farmers, regulators, and consumers of fresh produce are related to how, exactly, *Salmonella* is transferred between waterways and various crops, and what risk this carries for consumers. The potential for crop contamination caused by *Salmonella* from waterways is thought to be concentration-dependent, at least in part, and future studies may determine minimum *Salmonella* concentrations likely to lead to consumer illness for various crops, regions, and irrigation regimes (73). In the meantime, it is important to avoid pressuring farmers to use ground water instead of surface water or to adopt excessive contamination-prevention strategies that have not been supported by science-based evidence. Removing vegetated buffers around waterways, vegetated borders around fields, or forested areas near ponds may conflict with long-standing and well-research conservation practices and may prove detrimental to water quality in the long-term.

REFERENCES

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin P. 2011. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17:7–15.

2. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra R. 2011. Foodborne illness acquired in the United States—unspecified agents. Emerg Infect Dis 17:16–22.

3. Painter JA, Hoekstra RM, Ayers T, Tauxe R V, Braden CR, Angulo FJ, Griffin PM. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. Emerg. Infect. Dis. 19:407–15.

4. U.S. Department of Agriculture. 2011. Foodborne illness cost calculator. Online, accessed 4/25/2014. http://webarchives.cdlib.org/sw1tx36512/http://www.ers.usda.gov/data/foodborneillness/.

5. CDC. 2012. CDC features: Trends in foodborne illness in the United States. Online, accessed 4/25/2014. http://www.cdc.gov/Features/dsfoodnet2012/index.html.

6. CDC. 2014. List of selected multistate foodborne outbreak investigations. Online, accessed 4/25/2014. www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html.

7. Doolittle RF, Feng DF, Tsang S, Cho G, Little E. 1996. Determining divergence times of the major kingdoms of living organisms with a protein clock. Science 271:470–7.

8. McQuiston J, Herrera-Leon S, Wertheim B, Doyle J, Fields P, Tauxe R, Logsdon J. 2008. Molecular phylogeny of the Salmonellae: relationships among Salmonella species and subspecies determined from four housekeeping genes and evidence of lateral gene transfer events. J. Bacteriol. 190:7060–7.

9. Desai PT, Porwollik S, Long F, Cheng P, Wollam A, Bhonagiri-Palsikar V, Hallsworth-Pepin K, Clifton SW, Weinstock GM, McClelland M. 2013. Evolutionary Genomics of Salmonella enterica Subspecies. MBio 4:e00579–12.

10. Boyd EF, Wang FS, Whittam TS, Selander RK. 1996. Molecular genetic relationships of the salmonellae. Appl. Environ. Microbiol. 62:804–8.

11. Grimont, PAD, Weill F. 2007. Antigenic formulae of the Salmonella serovars, 9th ed. Online, accessed 4/25/2014. https://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089.

12. Koboldt, DC, Steinberg, KM, Larson, DE, Wilson, RK, Mardis E. 2013. The next-generation sequencing revolution and its impact on genomics. Cell 155:27–38.

13. Izumiya H, Terajima J, Yamamoto S, Ohnishi M, Watanabe H, Kai A et al. 2013. Genomic analysis of Salmonella enterica serovar Typhimurium Definitive Phage Type 104 [letter]. Emerg Infect Dis [online] 19:(http://dx.doi.org/10.3201/eid1905.121395).

14. Sarwari AR, Magder LS, Levine P, McNamara AM, Knower S, Armstrong GL, Etzel R, Hollingsworth J, Morris JG. 2001. Serotype distribution of Salmonella isolates from food animals after slaughter differs from that of isolates found in humans. J. Infect. Dis. 183:1295–9.

15. Jones TF, Ingram LA, Cieslak PR, Vugia DJ, Tobin-D'Angelo M, Hurd S, Medus C, Cronquist A, Angulo FJ. 2008. Salmonellosis outcomes differ substantially by serotype. J. Infect. Dis. 198:109–14.

16. CDC. 2011. National Enteric Disease Surveillance: Salmonella Annual Report, 2011 . Online, accessed 4/25/2014. http://www.cdc.gov/ncezid/dfwed/pdfs/salmonella-annual-report-appendices-2011-508c.pdf.

17. Maier, RM, Pepper, IL, Gerba C. 2000. Environmental Microbiology. Harcourt, San Diego, CA.

18. Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, Casadesús J, Platt DJ, Olsen JE. 2000. Host adapted serotypes of Salmonella enterica. Epidemiol. Infect. 125:229–55.

19. Winfield MD, Groisman EA. 2003. Role of Nonhost Environments in the Lifestyles of Salmonella and Escherichia coli. Appl. Environ. Microbiol. 69:3687–3694.

20. Hoelzer, K, Switt, AIM, Wiedmann M. 2011. Animal contact as a source of human non-typhoidal salmonellosis. Vet Res. 42 (online).

21. Jackson BR, Griffin PM, Cole D, Walsh K, Chai S. 2013. Outbreak-associated Salmonella enterica Serotypes and Food Commodities, United States, 1998–2008. Emerg Infect Dis 19 (online).

22. CDC. 2011. National Antimicrobial Resistance Monitoring System: Enteric Bacteria, 2011. Online, accessed 4/25/2014 http://www.cdc.gov/narms/pdf/2011–annual–report–na.

23. Jay-Russell M. 2013. What is the risk from wild animals in food-borne pathogen contamination of plants? CAB Rev. 8 (online).

24. Patrick ME, Mahon BE, Zansky SM, Hurd S, Scallan E. 2010. Riding in shopping carts and exposure to raw meat and poultry products. Food Prot. 6:1016–1200.

25. Jurado V, Ortiz-Martinez A, Gonzalez-delValle M, Hermosin B, Saiz-Jimenez C. 2002. Holy water fonts are reservoirs of pathogenic bacteria. Environ. Microbiol. 4:617–620.

26. Alvarez-Ordóñez A, Begley M, Prieto M, Messens W, López M, Bernardo A, Hill C. 2011. Salmonella spp. survival strategies within the host gastrointestinal tract. Microbiology 157:3268–81.

27. Giannella R a, Broitman S a, Zamcheck N. 1973. Influence of gastric acidity on bacterial and parasitic enteric infections. A perspective. Ann. Intern. Med. 78:271–6.

28. Álvarez-Ordóñez A, Prieto M, Bernardo A, Hill C, López M. 2012. The Acid Tolerance Response of Salmonella spp.: An adaptive strategy to survive in stressful environments prevailing in foods and the host. Food Res. Int. 45:482–492.

29. Blaser, MJ, Newman L. 1982. JSTOR: Reviews of Infectious Diseases, Vol. 4, No. 6 (Nov. - Dec., 1982), pp. 1096-1106. Rev. Infect. Dis. 4:1096–1106.

30. Dougan G, John V, Palmer S, Mastroeni P. 2011. Immunity to salmonellosis. Immunol. Rev. 240:196–210.

31. Teunis PFM, Kasuga F, Fazil A, Ogden ID, Rotariu O, Strachan NJC. 2010. Dose-response modeling of Salmonella using outbreak data. Int. J. Food Microbiol. 144:243–9.

32. Okoro CK, Kingsley RA, Connor TR, Harris SR, Parry CM, Al-Mashhadani MN, Kariuki S, Msefula CL, Gordon MA, de Pinna E, Wain J, Heyderman RS, Obaro S, Alonso PL, Mandomando I, MacLennan CA, Tapia MD, Levine MM, Tennant SM, Parkhill J, Dougan G. 2012. Intracontinental spread of human invasive Salmonella Typhimurium pathovariants in sub-Saharan Africa. Nat. Genet. 44:1215–21.

33. Kariuki S, Revathi G, Kariuki N, Kiiru J, Mwituria J, Muyodi J, Githinji JW, Kagendo D, Munyalo A, Hart CA. 2006. Invasive multidrug-resistant non-typhoidal Salmonella infections in Africa: zoonotic or anthroponotic transmission? J. Med. Microbiol. 55:585–91.

34. Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA. 2012. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. Lancet 379:2489–99.

35. Sanchez, S, Hofacre, CL, Lee, MD, Maurer, JJ, Doyle M. 2002. Animal sources of salmonellosis in humans. JAVMA 221:492–497.

36. Mulvey MR, Boyd D a, Finley R, Fakharuddin K, Langner S, Allen V, Ang L, Bekal S, El Bailey S, Haldane D, Hoang L, Horsman G, Louis M, Robberts L, Wylie J. 2013. Ciprofloxacin-resistant Salmonella enterica serovar Kentucky in Canada. Emerg. Infect. Dis. 19:999–1001.

37. Biggs HM, Lester R, Nadjm B, Mtove G, Todd JE, Kinabo GD, Philemon R, Amos B, Morrissey AB, Reyburn H, Crump JA. 2014. Invasive Salmonella Infections in Areas of High and Low Malaria Transmission Intensity in Tanzania. Clin. Infect. Dis. 58:638–647.

38. Cunnington AJ, Njie M, Correa S, Takem EN, Riley EM, Walther M. 2012. Prolonged neutrophil dysfunction after Plasmodium falciparum malaria is related to hemolysis and heme oxygenase-1 induction. J. Immunol. 189:5336–46.

39. US Food and Drug Administration. 2013. Standards for the growing, harvesting, packing, and holding of produce for human consumption. Online, accessed 4/25/2014. Fed. Regist. https://federalregister.gov/a/2013–00123.

40. Shelton DR, Karns JS, Coppock C, Patel J, Sharma M, Pachepsky YA. 2011. Relationship between eae and stx virulence genes and Escherichia coli in an agricultural watershed: implications for irrigation water standards and leafy green commodities. J. Food Prot. 74:18–23.

41. Harwood VJ, Levine AD, Scott TM, Chivukula V, Lukasik J, Farrah SR, Rose JB. 2005. Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. Appl. Environ. Microbiol. 71:3163–70.

42. Lewis Ivey ML, Miller SA. 2013. Assessing the efficacy of pre-harvest, chlorine-based sanitizers against human pathogen indicator microorganisms and Phytophthora capsici in non-recycled surface irrigation water. Water Res. 47:4639–4651.

43. US Department of Agriculture. 2011. Good Agricultural Practices and Good Handling Practices Audit Verification Program. Online, accessed 4/25/2014. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=s.

44. Center for Food Safety and Nutrition. 1998. Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables. Online, accessed 4/25/2014 http://www.fda.gov/downloads/Food/GuidanceComplian.

45. Food and Agriculture Organization of the United Nations. FAOSTAT Gateway. Online, accessed 4/25/2014. http://faostat3.fao.org/faostat–gateway/go/to/down.

46. Olaimat AN, Holley RA. 2012. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol. 32:1–19.

47. Jacobsen CS, Bech TB. 2012. Soil survival of Salmonella and transfer to freshwater and fresh produce. Food Res. Int. 45:557–566.

48. Johnson JYM, Thomas JE, Graham TA, Townshend I, Byrne J, Selinger LB, Gannon VPJ. 2003. Prevalence of Escherichia coli O157:H7 and Salmonella spp. in surface waters of southern Alberta and its relation to manure sources. Can. J. Microbiol. 49:326–35.

49. Marti R, Scott A, Tien Y-C, Murray R, Sabourin L, Zhang Y, Topp E. 2013. Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. Appl. Environ. Microbiol. 79:5701–9.

50. Dungan RS. 2010. Fate and transport of bioaerosols associated with livestock operations and manures. J. Anim. Sci. 88:3693–706.

51. US Food and Drug Administration. 2001. Analysis and evaluation of preventive control measures for the control & reduction/elimination of microbial hazards on fresh and fresh-cut produce. Online, accessed 4/25/2014 http://www.fda.gov/Food/FoodScienceResearch/SafePr.

52. Solomon EB, Yaron S, Matthews KR. 2002. Transmission of Escherichia coli O157:H7 from Contaminated Manure and Irrigation Water to Lettuce Plant Tissue and Its Subsequent Internalization. Appl. Environ. Microbiol. 68:397–400.

53. Erickson M. 2012. Internalization of fresh produce by foodborne pathogens. Annu. Rev. Food Sci. Technol. 3:283–310 http://www.annualreviews.org/doi/pdf/10.11.

54. Patel J, Millner P, Nou X, Sharma M. 2010. Persistence of enterohaemorrhagic and nonpathogenic E. coli on spinach leaves and in rhizosphere soil. J. Appl. Microbiol. 108:1789–96.

55. Kalwasińska A, Burkowska A. 2013. Municipal landfill sites as sources of microorganisms potentially pathogenic to humans. Environ. Sci. Process. Impacts 15:1078–86.

56. Withers PJ, Jordan P, May L, Jarvie HP, Deal NE. 2013. Do septic tank systems pose a hidden threat to water quality? Front. Ecol. Environ. 12:123–130.

57. Beam A, Garber L, Sakugawa J, Kopral C. 2013. Salmonella awareness and related management practices in U.S. urban backyard chicken flocks. Prev. Vet. Med. 110:481–8.

58. Refsum T, Handeland K, Baggesen DL, Holstad G, Kapperud G. 2002. Salmonellae in Avian Wildlife in Norway from 1969 to 2000. Appl. Environ. Microbiol. 68:5595–5599.

59. Gorski L, Parker CT, Liang A, Cooley MB, Jay-Russell MT, Gordus AG, Atwill ER, Mandrell RE. 2011. Prevalence, distribution, and diversity of Salmonella enterica in a major produce region of California. Appl. Environ. Microbiol. 77:2734–48.

60. Thomas JL, Slawson RM, Taylor WD. 2013. Salmonella serotype diversity and seasonality in urban and rural streams. J. Appl. Microbiol. 114:907–22.

61. Marcela F, Violeta B, Vanessa G, Pilar L, Natalia P, Pedro A, Alda F, Patricio R. 2013. Identification of diverse Salmonella Serotypes, Virulotypes, and Antimicrobial Resistance Phenotypes in Waterfowl From Chile. Vector-Borne Zoonotic Dis. 13:884–887.

62. Jensen AN, Lodal J, Baggesen DL. 2004. High diversity of Salmonella serotypes found in an experiment with outdoor pigs. NJAS - Wageningen J. Life Sci. 52:109–117.

63. Everard, COR, Tota, B, Bassett, D, Ali C. 1979. Salmonella in wildlife from Trinidad and Grenada, W.I. Wild. Dis. 15:213–219.

64. Mather AE, Reid SWJ, Maskell DJ, Parkhill J, Fookes MC, Harris SR, Brown DJ, Coia JE, Mulvey MR, Gilmour MW, Petrovska L, de Pinna E, Kuroda M, Akiba M, Izumiya H, Connor TR, Suchard MA, Lemey P, Mellor DJ, Haydon DT, Thomson NR. 2013. Distinguishable epidemics of multidrug-resistant Salmonella Typhimurium DT104 in different hosts. Science 341:1514–7.

65. Gennet S, Howard J, Langholz J, Andrews K, Reynolds MD, Morrison SA. 2013. Farm practices for food safety: an emerging threat to floodplain and riparian ecosystems. Front. Ecol. Environ. 11:236–242.

66. Alexandrov VA, Hoogenboom G. 2001. Climate variation and crop production in Georgia, USA, during the twentieth century. Clim. Res. 17:33–43.

67. Beretti M, Stuart D. 2008. Food safety and environmental quality impose conflicting demands on Central Coast growers. Calif. Agric. 62:68–73.

68. Balkcom G. 2010. Demographic Parameters of Rural and Urban Adult Resident Canada Geese in Georgia. Wildl. Manag. 74:120–123.

69. Warner C. 2013. Evaluation of various foliar sprays for use as a Canada goose grazing deterrent Masters thesis. Online, accessed 4/25/2014. http:/.

70. Cole D, Drum DJ V, Stalknecht DE, White DG, Lee MD, Ayers S, Sobsey M, Maurer JJ. 2005. Free-living Canada geese and antimicrobial resistance. Emerg. Infect. Dis. 11:935–8.

71. Haley BJ, Cole DJ, Lipp EK. 2009. Distribution, diversity, and seasonality of waterborne salmonellae in a rural watershed. Appl. Environ. Microbiol. 75:1248–55.

72. Pachepsky Y, Shelton DR, McLain JET, Patel J, Mandrell RE. 2011. Chapter Two – Irrigation Waters as a Source of Pathogenic Microorganisms in Produce: A Review, p. 75–141. *In* Advances in Agronomy.

73. Stine SW, Song I, Choi CY, Gerba CP. 2005. Application of Microbial Risk Assessment to the Development of Standards for Enteric Pathogens in Water Used To Irrigate Fresh Produce. J. Food Prot. 5:913–918.

74. McEgan R, Mootian G, Goodridge LD, Schaffner DW, Danyluk MD. 2013. Predicting Salmonella populations from biological, chemical, and physical indicators in Florida surface waters. Appl. Environ. Microbiol. 79:4094–105.

75. Strawn LK, Fortes ED, Bihn EA, Nightingale KK, Gröhn YT, Worobo RW, Wiedmann M, Bergholz PW. 2013. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. Appl. Environ. Microbiol. 79:588–600.

76. Duffy EA, Lucia LM, Kells JM, Castillo A, Pillai SD, Acuff GR. 2005. Concentrations of Escherichia coli and Genetic Diversity and Antibiotic Resistance Profiling of Salmonella Isolated from Irrigation Water, Packing Shed Equipment, and Fresh Produce in Texas. J. Food Prot. 1:70–79.

77. Gorski L, Parker CT, Liang A, Cooley MB, Jay-Russell MT, Gordus AG, Atwill ER, Mandrell RE. 2011. Prevalence, distribution, and diversity of Salmonella enterica in a major produce region of California. Appl. Environ. Microbiol. 77:2734–48.

78. Vereen E, Lowrance RR, Jenkins MB, Adams P, Rajeev S, Lipp EK. 2013. Landscape and seasonal factors influence Salmonella and Campylobacter prevalence in a rural mixed use watershed. Water Res. 47:6075–85.

79. Desmarais TR, Solo-Gabriele HM, Palmer CJ. 2002. Influence of Soil on Fecal Indicator Organisms in a Tidally Influenced Subtropical Environment. Appl. Environ. Microbiol. 68:1165–1172.

80. Ishii S, Yan T, Shively DA, Byappanahalli MN, Whitman RL, Sadowsky MJ. 2006. Cladophora (Chlorophyta) spp. harbor human bacterial pathogens in nearshore water of Lake Michigan. Appl. Environ. Microbiol. 72:4545–53.

81. California Food Emergency Response Team. 2008. Investigation of the Taco John's Escherichia coli O157:H7 Outbreak Associated with Iceberg Lettuce. Online, accessed 4/25/2014. http://www.cdph.ca.gov/pubsforms/Documents/fdb%20e.

82. Söderström A, Osterberg P, Lindqvist A, Jönsson B, Lindberg A, Blide Ulander S, Welinder-Olsson C, Löfdahl S, Kaijser B, De Jong B, Kühlmann-Berenzon S, Boqvist S, Eriksson E, Szanto E, Andersson S, Allestam G, Hedenström I, Ledet Muller L, Andersson Y. 2008. A large Escherichia coli O157 outbreak in Sweden associated with locally produced lettuce. Foodborne Pathog. Dis. 5:339–49.

83. Green, SK, Daly, ER, Talbot, EA, Demma, SH, Patel, NJ, Hill, TA, Walderhaug, MO, Hoekstra, RM, Lynch, MF, Painter J. 2008. Recurrent Multistate Outbreak of Salmonella Newport Associated with Tomatoes from Contaminated Fields, 2005. Epidemiol. Infect. 136:157–165.

84. CDC. 2008. Outbreak of Salmonella serotype Saintpaul infections associated with multiple raw produce items--United States, 2008. Morb. Mortal. Wkly. Rep. 57:929–34.

85. Cooley M, Carychao D, Crawford-Miksza L, Jay MT, Myers C, Rose C, Keys C, Farrar J, Mandrell RE. 2007. Incidence and tracking of Escherichia coli O157:H7 in a major produce production region in California. PLoS One 2:e1159.

86. Walters SP, Thebo AL, Boehm AB. 2011. Impact of urbanization and agriculture on the occurrence of bacterial pathogens and stx genes in coastal waterbodies of central California. Water Res. 45:1752–62.

87. Szonyi, B, Wade, SE, Mohammed H. 2010. Temporal and spatial dynamics of Cryptosporidium parvum infection on dairy farms in the New York City Watershed: a cluster analysis based on crude and Bayesian risk estimates. Int. J. Health Geogr. 9:(online). http://www.ij–healthgeograp hics.com/content/9/1/3.

88. Ferguson C, Husman AM de R, Altavilla N, Deere D, Ashbolt N. 2003. Fate and Transport of Surface Water Pathogens in Watersheds. Crit. Rev. Environ. Sci. Technol. 33:299–361.

89. Pachepsky YA, Shelton DR. 2011. Escherichia Coli and Fecal Coliforms in Freshwater and Estuarine Sediments. Crit. Rev. Environ. Sci. Technol. 41:1067–1110.

90. Rodgers P, Soulsby C, Hunter C, Petry J. 2003. Spatial and temporal bacterial quality of a lowland agricultural stream in northeast Scotland. Sci. Total Environ. 314-316:289–302.

91. Crim JF, Schoonover JE, Lockaby BG. 2012. Assessment of Fecal Coliform and Escherichia Coli Across a Land Cover Gradient in West Georgia Streams. Water Qual. Expo. Heal. 4:143–158.

92. Steele M, Odumeru J. 2004. Irrigation Water as Source of Foodborne Pathogens on Fruit and Vegetables. J. Food Prot. 12:2839–2849.

93. Luo Z. Distribution and Genetic Diversity of Salmonella Enterica Isolated from Irrigation Water in the Suwannee River Watershed. International Association for Food Protection, 2013 Annual Meeting. Online, accessed 4/25/2014. https://iafp.confex.com/iafp/2013/web.

94. Martin G. Discrete storm impacts on the loading of Salmonella and Campylobacters within a south Georgia rural watershed. 2009. Thesis, Univ. of Georgia. Online, accessed 4/25/2014. https://getd.libs.uga.edu/pdfs/martin_gordon_e_200905_ms.pdf.

95. CDC. An Atlas of Salmonella in the United States, 1968-2011. 2014. Online, accessed 4/25/2014. http://www.cdc.gov/salmonella/pdf/salmonella-atlas-508c.pdf.

96. Kenny, JF, Barber, NL, Hutson, SS, Linsey, KS, Lovelace, JK, Maupin M. Estimated Use of Water in the United States in 2005. USGS, Circular 1344. 2009. Online, accessed 4/25/2014 http://pubs.usgs.gov/circ/1344/pdf/c1344.pdf.

97. Ignatius A, Stallins JA. 2011. Assessing Spatial Hydrological Data Integration to Characterize Geographic Trends in Small Reservoirs in the Apalachicola-Chattahoochee-Flint River Basin. Southeast. Geogr. 51:371–393.

98. Chaney PL, Boyd CE, Polioudakis E. 2012. Number, size, distribution, and hydrologic role of small impoundments in Alabama. J. Soil Water Conserv. 67:111–121.

99. Hook, J, Conger S. Roles of Farm Ponds and Potential Impacts on Streams in the Coastal Plain of GA. 2008. Presentation. Online, accessed 4/25/2014. http://www.nespal.org/sirp/AWP/2008_SoGa_Ponds.pdf.

100. US Food and Drug Administration. Background on the FDA Food Safety Modernization Act (FSMA). 2011. Online, accessed 4/25/2014. http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm239907.htm.

101. Gorny, JR, Giclas, H, Gombas, D, Means, K et al. Commodity Specific Food Safety Guidelines for the Lettuce and Leafy Greens Supply Chain, 1st ed. 2006. Online, accessed 4/25/2014. http://www.fda.gov/downloads/Food/FoodSafety/Product-SpecificInformation/FruitsVegetablesJuices/GuidanceComplianceRegulatory.

102. Anonymous. Tomato Best Practices Manual. 2007. Online, accessed 4/25/2014. http://fshn.ifas.ufl.edu/Food-Safety/commodity/tomato/resources/5G%20Tomato%20Best%20Practices%20Manual.pdf.

103. Gu G. Regional risks for Salmonella spp., Escherichia coli O157:H7 and Campylobacter jejuni contamination. International Association for Food Protection, 2012 Annual Meeting. Online, accessed 4/25/2014. https://iafp.confex.com/.

104. Spector MP, Kenyon WJ. 2012. Resistance and survival strategies of Salmonella enterica to environmental stresses. Food Res. Int. 45:455–481.

105. Vereen E. Defining Linkages Between Land-Use, Salmonella, and Campylobacter in the Satilla River Basin (Georgia, USA). 2010. Dissertation, Univ. of Georgia. Online, accessed 4/25/2014. https://getd.libs.uga.edu/pdfs/vereen_ethell_201012_phd.pdf.

106. Hernández-Reyes C, Schikora A. 2013. Salmonella, a cross-kingdom pathogen infecting humans and plants. FEMS Microbiol. Lett. 343:1–7.

107. Wenger S. A review of the scientific literature on riparian buffer width, extent, and vegetation. 1999. Online, accessed 4/25/2014. https://www.cc.utexas.edu/.

108. Natural Resources Conservation Service. 2010. Conservation Practice Standard. Online, accessed 4/25/2014. http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs143_026183.pdf.

109. US EPA. Level II Ecoregions. Online, accessed 4/25/2014. http://www.epa.gov/wed/pages/ecoregions/na_eco.htm#Level%20II.

110. Bosch DD, Sheridan JM, Marshall LK. 2007. Precipitation, soil moisture, and climate database, Little River Experimental Watershed, Georgia, United States. Water Resour. Res. 43:W09472.

111. Sheridan JM. 1997. Rainfall-streamflow relations for Coastal Plain watersheds. Appl. Eng. Agric. 13:333–344.

112. Sullivan, DG, Batten, HL, Bosch, D, SHeridan, J, Strickland T. 2007. Little river experimental watershed, Tifton, Georgia, United States: A geographic database. Water Resour. Res. 43:W09475.

113. Lowrance R, Vellidis G. 1995. A conceptual model for assessing ecological risk to water quality function of bottomland hardwood forests. Environ. Manage. 19:239–258.

114. Lowrance R, Todd R, Fail, J, Hendrickson, O, Leonard R, Asmussen L. 1984. Riparian Forests as Nutrient Filters in Agricultural Watersheds. Bioscience 34:374–377.

115. Bosch, DD, Sheridan, JM, Davis F. 1999. Rainfall characteristics and spatial correlation for the Georgia coastal plain. Trans. Am. Soc. Agric. Biol. Eng. 42:1637–1644.

116. Jang T, Vellidis G, Hyman JB, Brooks E, Kurkalova LA, Boll J, Cho J. 2013. Model for prioritizing best management practice implementation: sediment load reduction. Environ. Manage. 51:209–24.

117. Cho J, Vellidis G, Bosch DD, Lowrance R, Strickland T. 2010. Water quality effects of simulated conservation practice scenarios in the Little River Experimental watershed. J. Soil Water Conserv. 65:463–473.

118. USDA-NASS. 2013. USDA National Agricultural Statistics Service Cropland Data Layer. 2013. Online, accessed 4/25/2014. http://nassgeodata.gmu.edu/CropScape/.

119. NRCS. Watershed Boundary Dataset. 2013. Online, accessed 4/25/2014. http://datagateway.nrcs.usda.gov.

120. NRCS. National Aerial Imagery Program. 2011. Online, accessed 4/25/2014. http://datagateway.nrcs.usda.gov/.

121. Gu G, Luo Z, Cevallos-Cevallos JM, Adams P, Vellidis G, Wright A, van Bruggen AHC. 2013. Factors affecting the occurrence of Escherichia coli O157 contamination in irrigation ponds on produce farms in the Suwannee River Watershed. Can. J. Microbiol. 59:175–82.

122. Gu G, Luo Z, Cevallos-Cevallos JM, Adams P, Vellidis G, Wright A, van Bruggen AHC. 2013. Occurrence and population density of Campylobacter jejuni in irrigation ponds on produce farms in the Suwannee River Watershed. Can. J. Microbiol. 59:339–46.

123. US EPA. Method 1682: Salmonella in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium. 2006. Online, accessed 4/25/2014. http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2008_11_25_methods_method_biological_1682.pdf.

124. US FDA. 2014. Bacteriological Analytical Manual: Salmonella. 2014. Online, accessed 4/25/2014 http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm.

125. Chiu CH, Ou JT. 1996. Rapid identification of Salmonella serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture-multiplex PCR combination assay. J. Clin. Microbiol. 34:2619–22.

126. Jarvis B, Wilrich C, Wilrich P-T. 2010. Reconsideration of the derivation of Most Probable Numbers, their standard deviations, confidence bounds and rarity values. J. Appl. Microbiol. 109:1660–7.

127. US FDA. Bacteriological Analytical Manual Appendix 2: Most Probable Number from Serial Dilutions. 2010. Online, accessed 4/25/2014 http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm.

128. US EPA. Guidelines establishing test procedures for the analysis of pollutants. 2014. Online, accessed 4/25/2014. http://www.ecfr.gov/. Electronic Code of Federal Regulations., Washington, DC.

129. American Public Health Association. 2005. Standard Methods for the Examination of Wastewater, Method 2540 D.Standard methods for the examination of water and wastewater., 18th ed. American Public Health Association, Washington, DC.

130. University of Georgia-Griffin. Georgia Weather - Automated Environmental Monitoring Network. 2014. Online, accessed 4/25/2014. http://www.georgiaweather.net/.

131. Clarke JU. 1998. Evaluation of Censored Data Methods To Allow Statistical Comparisons among Very Small Samples with Below Detection Limit Observations. Environ. Sci. Technol. 32:177–183.

132. Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24:127–35.

133. Bates D. 2010. lme4: Mixed-effects modeling with R. Online, accessed 4/25/2014. http://lme4.r-forge.r-project.org/lMMwR/lrgprt.pdf.

134. Bates, D, Maechler, M, Bolker, B, Walker S. lme4: Linear mixed-effects models using Eigen and S4. 2013. Online, accessed 4/25/2014. CRAN.R-project.org/package=lme4.

135. R Core Team. R: A language and environment for statistical computing. 2013. Available online, accessed 4/25/2014. www.R-project.org. R Foundation for Statistical Computing, Vienna, Austria.

136. Nieuwenhuis, Rense, te Grotenhuis, Manfred, Pelzer B. 2012. Influence.ME: Tools for Detecting Influential Data in Mixed Effects Models. R Journal, 4(2): 38–47.

137. Frederick, RH, Myers, VA, Auciello E. 1977. NOAA Technical Memorandum NWS HYDRO-35: Five- to 60-minute precipitation frequency for the eastern and central United States. National Oceanic and Atmospheric Administration and the National Weather Service.

138. Galland, JC, Troutt, HF, Brewer R et al. 2001. Diversity of Salmonella serotypes in cull (market) dairy cows at slaughter. Vet. Med. Today 219:1216–1220.

139. Ndiaye, ML, Dieng, Y, Niang, S, Pfeifer, HR, Tonolla, M, Peduzzi R. 2011. Effect of irrigation water on the incidence of Salmonella spp. on lettuces produced by urban agriculture and sold on the markets in Dakar, Senegal. African J. Microbiol. Res. 5:2885–2890.

140. Santiago-Rodriguez TM, Tremblay RL, Toledo-Hernandez C, Gonzalez-Nieves JE, Ryu H, Santo Domingo JW, Toranzos GA. 2012. Microbial quality of tropical inland waters and effects of rainfall events. Appl. Environ. Microbiol. 78:5160–9.

141. Ackerman, D, Weisberg S. 2003. Relationship between rainfall and beach bacterial concentrations on Santa Monica Bay beaches. J. Water Heal. 1:85–89.

142. Walsh CJ, Roy AH, Feminella JW, Cottingham PD, Groffman PM, Morgan RP. 2005. The urban stream syndrome: current knowledge and the search for a cure. J. North Am. Benthol. Soc. 24:706.

143. Patchanee P, Molla B, White N, Line DE, Gebreyes WA. 2010. Tracking salmonella contamination in various watersheds and phenotypic and genotypic diversity. Foodborne Pathog. Dis. 7:1113–20.

144. Sidhu JPS, Hodgers L, Ahmed W, Chong MN, Toze S. 2012. Prevalence of human pathogens and indicators in stormwater runoff in Brisbane, Australia. Water Res. 46:6652–60.

145. Sassoubre LM, Walters SP, Russell TL, Boehm AB. 2011. Sources and fate of Salmonella and fecal indicator bacteria in an urban creek. J. Environ. Monit. 13:2206–12.

146. Parker WF, Mee BJ. 1982. Survival of Salmonella adelaide and fecal coliforms in coarse sands of the swan costal plain, Western Australia. Appl. Envir. Microbiol. 43:981–986.

147. Aminabadi P. Evaluation of Foodborne Pathogens in Aquatic Wildlife and Irrigation Ponds in Southeastern Georgia. International Association for Food Protection, 2013 Annual Meeting. Presentation. Online, accessed 4/25/2014. https://iafp.confex.com/iafp/2013/webprogram/.

148. Proctor ME, Hamacher M, Tortorello ML, Archer JR, Davis JP. 2001. Multistate outbreak of Salmonella serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. J. Clin. Microbiol. 39:3461–5.

149. CDC. 2009. Outbreak of Salmonella Serotype Saintpaul Infections Associated with Eating Alfalfa Sprouts — United States, 2009. Morb. Mortal. Wkly. Rep. 58:1–4.

150. Woodward D, Khakhria R, Johnson W. 1997. Human salmonellosis associated with exotic pets. J. Clin. Microbiol. 35:2786–2790.

151. CDC. Multistate Outbreak of Salmonella Bareilly and Salmonella Nchanga Infections Associated with a Raw Scraped Ground Tuna Product (Final Update). 2012. Online, accessed 4/25/2014. http://www.cdc.gov/salmonella/bareilly-04-12/index.html.

152. Guo X, Chen J, Brackett RE, Beuchat LR. 2001. Survival of Salmonellae on and in Tomato Plants from the Time of Inoculation at Flowering and Early Stages of Fruit Development through Fruit Ripening. Appl. Environ. Microbiol. 67:4760–4764.

153. Mahon BE, Pönkä A, Hall WN, Komatsu K, Dietrich SE, Siitonen A, Cage G, Hayes PS, Lambert-Fair MA, Bean NH, Griffin PM, Slutsker L. 1997. An International Outbreak of Salmonella Infections Caused by Alfalfa Sprouts Grown from Contaminated Seeds. J. Infect. Dis. 175:876–882.

154. Seixas R, Machado J, Bernardo F, Vilela C, Oliveira M. 2014. Biofilm Formation by Salmonella Enterica Serovar 1,4,[5],12:i:- Portuguese Isolates: A Phenotypic, Genotypic, and Socio-geographic Analysis. Curr. Microbiol. 68:670–7.

155. Hanning IB, Nutt JD, Ricke SC. 2009. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodborne Pathog. Dis. 6:635–48.

156. Hilbert F, Smulders FJM, Chopra-Dewasthaly R, Paulsen P. 2012. Salmonella in the wildlife-human interface. Food Res. Int. 45:603–608.

157. Berger CN, Sodha S V, Shaw RK, Griffin PM, Pink D, Hand P, Frankel G. 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ. Microbiol. 12:2385–97.

158. Wheatley M. 2010. Domains of scale in forest-landscape metrics: Implications for species-habitat modeling. Acta Oecologica 36:259–267.

159. Higgins JA, Belt KT, Karns JS, Russell-Anelli J, Shelton DR. 2005. tir- and stx-positive Escherichia coli in stream waters in a metropolitan area. Appl. Environ. Microbiol. 71:2511–9.

160. Harris JR, Neil KP, Behravesh CB, Sotir MJ, Angulo FJ. 2010. Recent multistate outbreaks of human salmonella infections acquired from turtles: a continuing public health challenge. Clin. Infect. Dis. 50:554–9.

161. Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, Koehler J, Marcus R, Angulo FJ. 2004. Reptiles, amphibians, and human Salmonella infection: a population-based, case-control study. Clin. Infect. Dis. 38 Suppl 3:S253–61.

162. Kilonzo C, Li X, Vivas EJ, Jay-Russell MT, Fernandez KL, Atwill ER. 2013. Fecal shedding of zoonotic food-borne pathogens by wild rodents in a major agricultural region of the central California coast. Appl. Environ. Microbiol. 79:6337–44.

163. Lowrance R, Todd R, Fail J, Hendrickson O, Leonard R, Amussen L. 1984. Riparian Forests as Nutrient Filters in Agricultural Watersheds. Bioscience 34:374–7.

164. Georgia Soil and Water Conservation Commission. 2007. Best Management Practices for Georgia Agriculture. Available online accessed 4/8/2014. http://www. gaepd. org/.

165. Dillaha, TA. 1986. Long-Term Effectiveness and Maintenance of Vegetative Filter Strips. Available online, accessed 4/28/2014. http://lshs.tamu.edu/.

166. Mendez A, Dillaha TA, Mostaghimi S. 1999. Sediment and Nitrogen Transport in Grass Filter Strips. J. Am. Water Resour. Assoc. 35:867–875.

167. Muñoz-Carpena R, Parsons JE, Gilliam JW. 1999. Modeling hydrology and sediment transport in vegetative filter strips. J. Hydrol. 214:111–129.