ABSTRACT

Title of Thesis:	SPATIAL AND TEMPORAL VARIANCE OF MICROBIAL WATER QUALITY IN TWO MARYLAND IRRIGATION PONDS
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Farm ponds must be regularly sampled for *Escherichia coli* (*E. coli*) concentrations to evaluate the health risks of using pond water for irrigation. However, no guidance is available regarding sampling locations and/or irrigation pump placement. We hypothesized that there exists spatial and/or temporal patterns of *E. coli* concentrations across ponds. To test this hypothesis, we sampled two irrigation ponds in Maryland biweekly during the summers of 2016 and 2017. Results from data analysis of mean relative differences and Spearman correlation coefficients are presented. Empirical orthogonal functions indicated spatial patterns of Log *E. coli* concentrations were temporally maintained. More sample variance existed over time in the pond interiors versus near shore locations. Furthermore, larger patterns of sample variance existed within the spatial analysis variance versus the temporal analysis variance over both ponds for this

study. Therefore, the spatio-temporal *E. coli* variance may have significant impacts on sampling and pump intake locations.

SPATIAL AND TEMPORAL VARIANCE OF MICROBIAL WATER QUALITY IN TWO MARYLAND IRRIGATION PONDS

by

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Preface

This thesis was written in a journal style and organized into three chapters and followed by a conclusions section. The chapters represent different years of research and each is comprised of an introduction, materials and methods, results, conclusion, and literature cited sections. Each chapter is intended for separate publication; therefore, repetition is present in some sections.

Dedication

This thesis is dedicated to my parents, Michael and Tina Kierzewski.

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I am very grateful to all of those who have helped me during my time as a graduate student. Thank you to the people at University of Maryland as well as personnel from the USDA Environmental Microbial Food Safety Laboratory for providing me with funding and guidance for this project. Thank you to Dr. Yakov Pachepsky for project development and funding, and data analysis guidance. Thank you to Matthew Stocker, Jackie Smith, and Billie Griffith for field work and sample processing assistance. Thank you to Dr. William Bowerman for his support and guidance.Thank you to Dr. Robert Hill for his unrelenting support and dedication to my success. Thank you to my committee members Dr. Lance Yonkos and Dr. Stephanie Yarwood for their encouragement and support.

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Chapter 1: Introduction and Literature Review

Foodborne Illnesses in the United States

The Center for Disease Control (CDC) defines foodborne illness as an illness in which epidemiological analysis deems food as a source (Centers for Disease Control and Prevention, 2011). There are several causes for food borne illness including chemical contamination, parasites, viruses and bacterial agents. Based on an analysis of data from 2000-2008, bacterial agents are the second leading cause of foodborne illnesses in the United States comprising approximately 39% of the total 9.4 million annual illnesses (Scallan et al., 2011). Most illnesses were attributed to norovirus followed by *Salmonella*, *Clostridium perfringens*, and *Campylobacter spp*. The US Department of Agriculture's Economic Research Service estimates the cost of foodborne illnesses, defined as medical treatment and loss of income, as approximately $15 billion a year (USDA web article Hoffman, 2017). The pathogen resulting in the greatest costs is *Shigella* followed by *Vibrio parahaemolyticus*, *Norovirus* and *Salmonella* as the top four.

With the increased integration of commercialized food production systems and the growing demand for constantly-available fresh produce, food borne illness outbreaks have spread reaching a larger portion of the world population (Lynch et al., 2009). As an example, the CDC reports that an estimated 9.4 million cases of foodborne illness occur annually in the United States alone (Scallan et al., 2011). Norovirus causes the largest portion of illnesses followed by *Salmonella*, *Clostridium perfringens*, *campylbactor spp*., and *staphylococcus aureus*. Although *E. coli* occurs

extensively throughout the environment, the *E. coli* (STEC) 0157 sub-species is one of the top five pathogens resulting in hospitalizations. Within the last 100 years, outbreaks have shifted from their traditional vehicles of transmission including dairy and meat to fresh fruit and vegetables due to increasing consumer demands (Sivapalasingam et al., 2004). From 1973 to 1997, a total of 190 produce-associated food-borne outbreaks resulted in 16,058 reported illnesses (Sivapalasingam et al. 2004). Produce alone accounted for 0.7% of the total outbreaks in the 1970s and 6% of the outbreaks in the 1990s. Painter et al. (2013) reported that 46% of illnesses were attributed to produce in 1998-2008. Possible reasons for the increased produce-related outbreaks included an overall increase of per capita produce consumption in the US, and the increased proximity of agricultural produce fields to animal-based farms and wild animal habitats (Lynch et al., 2009). Contamination may potentially occur at any step in the food production process from use of unsanitary irrigation water to the improper cleansing of market produce. Irrigation water quality as a source of fecal contamination will be the focus of this discussion.

Surface irrigation water as a source of foodborne pathogens

Irrigation used in produce operations may be fresh surface water and/or subsurface water. The surface water more so than the subsurface water may be a potential source of fecal contamination and includes ponds, streams, rivers, and lakes. In 2016, 10 irrigation ponds in southern Georgia and northern Florida were tested for concentrations of *Salmonella enterica* and generic *Escherichia coli* (Antaki et al., 2016). The study found that all the ponds contained *Salmonella* and 28.2% of the total samples had a geometric mean concentration of 0.26 most probable number

[MPN]/liter. These concentrations of *Salmonella* were highly correlated with both temperature and rainfall.

Surface waters are highly susceptible to contamination from point sources or pollutants that are carried during hydrological events. The highest pathogen concentrations in surface waters typically occur following rainfall events (Gerba, 2009). Wachtel et al. (2002) reported a sewage spill was released into a creek in 2000 that was unknowingly used for the irrigation of cabbage with the unchlorinated tertiary-treated effluent. *E. coli* was subsequently isolated from the cabbage roots, but not the cabbage heads themselves. Despite the risk, surface water is becoming increasingly utilized due to the over extraction of groundwater in some regions (Uyttendaele et at. 2015).

From 2003 to 2008, the number of US farms using only groundwater for irrigation decreased by 9.2% while the use of surface water use increased by 6.3% (Pachepsky et al., 2011). The growing trend of small farm production has spurred the use of surface waters while at the same time some of the largest outbreaks of food borne illnesses in the US have been attributed to the use of contaminated irrigation water. Two multistate outbreaks of *Salmonella Newport* that occurred in 2005 were traced back to irrigation ponds in Virginia (Greene et al., 2008). Approximately 52 residents in Montana were infected by *Escherichia coli* 0157:H7 during 1998 that was attributed to the consumption of leaf lettuce (Achers et al., 1998). It was determined the leaf lettuce had been exposed to contaminated irrigation water that was the probable source of the outbreak. Even more recently in December 2018, romaine lettuce from farms in Santa Barbara County, California harbored *E. coli* 0157: H7

isolates that caused illness in 16 states with 62 reported cases of food-borne illness (CDC Food Safety Alert, 2019). Scientists from the Food and Drug Administration (FDA) and the CDC found samples of the *E. coli* 0157:H7 isolates in sediments collected from the agricultural water reservoirs used by the lettuce producers. It is obvious that irrigation water provides a vehicle for the transmission of foodborne pathogens. In response to the increased occurrence of these outbreaks, suggestive preventative measures and outbreak management has primarily been used to mitigate the spread and prevalence of these recent instances of food-borne illness.

Fecal Indicator Bacteria (FIB)

The need for readily monitoring and regulating for the presence of pathogens in water sources has prompted the routine testing for fecal indicator bacteria (FIB). Fecal indicator bacteria are readily found throughout the environment since they thrive in abundance within the gastrointestinal track of humans and other mammals (Haack USGS, 2017) Fecal bacteria are typically harmless, however some fecal bacterial strains are pathogenic and may cause disease in humans. These pathogenic FIB strains are transmitted, just like all other fecal bacteria, through the feces of wild or domesticated farm animals and humans. Infection from pathogenic strains present symptoms in the form of diarrhea, abdominal cramps, and may lead to a uremic hemolytic syndrome that can be a serious illness (Mayo Clinic *E. coli* Fact Sheet). The testing for specific pathogens is oftentimes expensive and time-consuming since parasites, viruses, bacteria and protozoan may all serve as possible causes. The FIB may easily be measured using simple laboratory cultivation and isolation methods.

Because of the simplicity and low cost of testing, FIB are commonly used as a surrogate for pathogenic organisms that may also spread from fecal contamination. Since 1986, the US Environmental Protection Agency (EPA) has supported the use of FIB in monitoring the sanitary water quality for recreational, agricultural and potable water sources (USEPA Ambient Water Quality for Bacteria, 1986). Fecal indicator bacteria include *Salmonella*, *Campylobactor*, *Entercoccus*, and *E. coli*. Based on research conducted in 1972, the EPA reported that *E. coli* had the strongest correlation with illnesses in freshwater environments out of several FIBs that were studied (USEPA Ambient Water Quality for Bacteria, 1986). Today, *E. coli* concentrations are used as a criterion in water quality guidelines and regulations despite the criticisms of some questioning the analysis of the 1972 study (Moore, 1986; Henderson, 1975; Cabelli, 1975) and a more recent study that questions the appropriateness of using *E.coli* concentrations as the only dominant criterion for water quality purity (Pachepsky et al., 2016).

Guidelines and Regulations of Microbial Water Quality in United States Irrigation Ponds

USDA Good Agricultural Practices (GAP) & Good Handling Practices (GHP)

Before specific water quality regulations were established, suggested guidelines were put into place to begin the focus on water quality management. President Clinton began a new Food Safety Initiative in 1997 which prompted the FDA and USDA to establish the "Guidance for Industry-Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables" (FDA Guide to

minimize microbial food safety hazards for fresh fruits and vegetables, 1997). The guide suggested that farmers should focus on good agricultural practices and may elect to test their water for microbial contamination. In response to the FDA 1997 guide, the USDA created a way for farmers to be held accountable for their good practices. The Good Agricultural Practices and Good Handling Practices Audit program verified if farmers were conforming to the suggested guidelines (USDA GAP &GHP Audit Program, 2002). This Program set testing frequencies for surface waters at 3 times during a growing season, before planting, at growing season peak and near harvest. Maximum microbial levels were suggested using the EPA Recreational Water Standards and the US-EPA Drinking Water Standards.

History of Recreational Water Quality Proposed Regulations

In order to formally regulate water quality, research was conducted to establish limits on microbial loading. The National Technical Advisory Committee of the US Department of the Interior suggested microbial criterion for bathing waters based on studies conducted from 1940-1950 (USEPA Ambient Water Quality for Bacteria, 1986). Bathing beaches in Illinois, Kentucky and New York were routinely monitored for water quality and the abundance of gastrointestinal illness. The study found that individuals who swam in fresh waters with a total coliform density of 2300 coliforms/ 100ml had significantly higher illness rates. In the 1960s the regulations transitioned to using less variable fecal coliform concentrations. Since only 18% of the total coliforms were fecal, they calculated that 400 fecal coliforms per 100ml correlated with the elevated illness rates. The upper limitation for fecal coliform

bacteria was set at a log mean of 200/100ml with 90% of the samples under 400/100ml in any 30-day period. In 1972, EPA began a series of studies in fresh water and marine bathing beaches which were designed to correct the deficiencies of the original 1968 studies in Illinois and Kentucky (USEPA Ambient Water Quality for Bacteria, 1986). Marine and freshwater beaches were selected encompassing sites with little to no contamination and other sites with high amounts of contamination. The study concluded that rates of swimming associated gastroenteritis were statistically significant in the more polluted beaches while the relatively unpolluted beaches did not have statistically significant rates of illness. The findings displayed a more detailed association between untreated sewage contaminated water and health risks for swimmers. The study concluded that a maximum geometric mean of 200/100ml of fecal indicator bacteria correlated with 8 illnesses per 1,000 swimmers in fresh water beaches. This research provided the groundwork for the water quality criterion we have today. (USEPA Ambient Water Quality for Bacteria, 1986)

The current use of the United States Food and Drug Administration Food Safety Modernization Act

The Food Safety Modernization Act (FSMA) is one of the only established formal regulatory laws setting standards for irrigation water quality in the United States. The initial version of the law was passed in the US House of Representatives on June 9, 2009. The title of the act was changed to FSMA and signed into law in 2011 (US Congress FDA FSMA 2011). Within the FSMA, the Produce Safety Rule

specifies regulations for surface irrigation water based on two numerical criteria. The first criterion is the geometric mean (GM) concentration of generic *E. coli* in a water source, which is the central tendency of the water quality. The rule states that the GM of samples in an irrigation water body must be less than 126 colony forming units (CFU) per 100 ml of water. The second criterion is the statistical threshold value (STV), which shows the variability of water quality under adverse conditions, such as high rainfall or high-water stream flow. Log STV is calculated using the GM + 1.282 (constant value) *std (log values). This level may be described as the threshold value with 90% of the sample *E. coli* concentrations being below the value. The statistical threshold value limitation is given as 410 CFU or less of generic *E. coli* in 100ml of water (U.S. Congress, 2011; Federal Register, 2015). An initial survey of an irrigation source should consist of at least 20 samples which are used to calculation GM and STV. An additional 5 samples should be taken annually to update the initial calculations. Despite the clear criterion for microbial water quality, the regulation lacks information on when and where to sample within a water source.

Research on Spatial and Temporal Variability in Microbial Water Quality

Possible Factors influencing E. coli density variability spatially and temporally

Microbial concentrations can vary based on seasonality, temperature, hydrological events, and land use (Wu et al., 2011; Reeves et al., 2004; Traister &Anisfeld, 2006; Quilliam et at., 2011). Wu et al 2011 found that based on Spearman

Rank Correlations, precipitation was significantly correlated with *E. coli* concentration densities. The *E. coli* concentrations and diversity seemed to vary in the wet and dry seasons due to the abundance of the fauna species. Urban areas comprised of forest had some of the lowest *E. coli* concentration densities perhaps due to lower human populations. A similar study found that bird abundances among other factors modified fecal bacteria concentrations (Shellenbarger et al., 2008). The *E. coli* concentrations may vary across years, seasons, and even within the same water body on a given day (Schilling et al., 2009; Whittman et al., 2008; Meays et al., 2006). Pond outflows have been found to have significantly lower *E. coli* concentrations than inflows which was attributed to the prolonged exposure of UV radiation (Jenkins et al., 2012). Quilliam et al. (2011) noticed that the spatial variation of *E. coli* concentrations was very different from one side of the River Conwy in the UK to the other side of the river. In order to account for the variability in *E. coli* concentrations, numerous samples of irrigation water must be taken throughout a growing season. To obtain this type of data requires the intensive and costly processing of samples. It would be beneficial if it were possible to correlate *E. coli* concentrations with a more easily measurable environmental covariate.

Bacteria and Phytoplankton/Cyanobacteria Interactions

Algae and cyanobacteria may be easily estimated using remote sensing (Richardson, 1996) and have been found to correlation with the *E. coli* concentrations in water. One such correlation was documented at Wisconsin beaches where large mats of *Cladophora* were harboring *E. coli* and promoting their extended survival

(Englebert et al., 2008). There were three waterborne illness outbreaks in 2001 and 2002 attributed to contaminated recreational waters of the lake. It was suggested that *Cladophora* might have provided a favorable environment by reducing the bacteria-limiting environmental factors such as heat and light stress. The *E. coli* within the mats may have become dislodged and permeated through the water to the surrounding areas. A microcosm experiment conducted later indicated that *E. coli* survival was highest when attached to *Cladophora*. A similar experiment by Byappanahalli et al. (2003) showed that algae leachate contained important nutrient and chemicals to promote *E. coli* growth. The bacterium, *Legionaella pneumophila*, that causes Legionnaires disease, was found to be naturally occurring with cyanobacteria mats composed of *Fischerella sp*., *Phormidium sp*., and *Oscillatoria sp.* (Tison et al., 1980). In the laboratory, *L. pneumophila* growth was found to be dependent on *Fischerella sp.* photosynthesis and substrate release. This mutualistic relationship was facilitated when algal photosynthesis released oxygen for *E. coli* respiration. Mating *Chlamydomanos reinhardii* released soluble carbohydrates allowing for the digestion of the cell wall and fusion of gametes (Cole, 1982). These carbohydrates were in turn consumed by heterotrophic bacteria. Dead algal cells can also be metabolized by bacteria. After high densities of fecal coliforms were observed in alpine streams within Wyoming, it was reported that *Chlorella* isolated from the streams released organic compounds that promoted bacterial growth (Cole, 1982). Positive correlations between bacterial and algal biomass have also been noted (Carr and Chambers, 2005). Knowledge of algae/cyanobacteria and *E. coli* interaction mechanisms appear to be

beneficial to interpret relationships between populations of those organisms in irrigation water sources.

Thesis overview

This thesis is composed of two principle chapters. Chapter 2 presents data from field studies conducted on two Maryland irrigation ponds during the growing season of 2016. Water *E. coli* concentrations as well as environmental covariates were measured to understand the spatial and temporal distribution of microbial concentrations. Additional water samples were collected to measure chlorophyll-a concentrations. The study tested two hypotheses: 1) in a given irrigation pond there is a spatial pattern in *E. coli* concentrations that is preserved during the irrigation season, and 2) algae and cyanobacteria populations in the form of chlorophyll-a concentrations are positively correlated with *E. coli* concentrations within the irrigation ponds studied. Data were analyzed using mean relative differences and Spearman Rank Correlations.

Chapter 3 focuses on whether trends of spatial and temporal distributions within *E. coli* concentrations are conserved from one year to the next or exhibit the same annual variance patterns. The same two ponds were sampled during the growing season of 2017. Sampling techniques were the same from the 2016 sampling year although additional water samples were taken in 2017. Data was analyzed using the mean relative differences and Spearman Rank Correlations. The data for 2016 and 2017 were also analyzed using the Empirical Orthogonal Functions (EOF) to examine spatial and temporal variance patterns within the dimensional data.

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Chapter 2-Temporal Stability of Escherichia coli Concentrations in Waters of Two Irrigation Ponds in Maryland

Abstract

Fecal contamination of water sources is an important water quality issue for agricultural irrigation ponds. *Escherichia coli* concentrations are commonly used to evaluate recreational and irrigation water quality. We hypothesized that there may exist temporally stable spatial patterns of *E. coli* concentrations across ponds, meaning that some areas mostly have higher and other areas mostly lower than average concentrations of *E. coli*. To test this hypothesis, we sampled two irrigation ponds in Maryland at nodes of spatial grids biweekly during the summer of 2016. Environmental covariates—temperature, turbidity, conductivity, pH, dissolved oxygen, chlorophyll *a*, and nutrients—were measured in conjunction with *E. coli* concentrations. Temporal stability was assessed using mean relative differences between measurements in each location and averaged measurements across ponds. Temporally stable spatial patterns of *E. coli* concentrations and the majority of environmental covariates were expressed for both ponds. In the pond interior, larger relative mean differences in chlorophyll *a* corresponded to smaller mean relative differences in *E. coli* concentrations, with a Spearman's rank correlation coefficient of 0.819. Turbidity and ammonium concentrations were the two other environmental covariates with the largest positive correlations between their location ranks and the *E. coli* concentration location ranks. Tenfold differences were found between geometric mean *E. coli* concentrations in locations that were consistently high or consistently low. The existence of temporally stable patterns of *E. coli* concentrations

can affect the results of microbial water quality assessment in ponds and should be accounted for in microbial water quality monitoring design.

Importance

The microbial quality of water in irrigation water sources must be assessed to prevent the spread of microbes that can cause disease in humans because of produce consumption. The microbial quality of irrigation water is evaluated based on concentrations of *Escherichia coli* as the indicator organism. Given the high spatial and temporal variability of *E. coli* concentrations in irrigation water sources, recommendations are needed on where and when samples of water have to be taken for microbial analysis. This work demonstrates the presence of a temporally stable spatial pattern in the distributions of *E. coli* concentrations across irrigation ponds. The ponds studied had zones where *E. coli* concentrations were mostly higher than average and zones where the concentrations were mostly lower than average over the entire observation period, covering the season when water was used for irrigation. Accounting for the existence of such zones will improve the design and implementation of microbial water quality monitoring.

Introduction

The microbial quality of irrigation water has recently attracted substantial attention. Approximately 76 million people in the United States become ill from foodborne diseases annually, and over 40% of these cases are linked to fresh produce (Painter et al. 2013). Irrigation water can be a significant source of pathogenic microorganisms in produce, and hence, assessing potential contamination from water

sources is important for human and animal health (Wood et al., 2010; Gelting et al., 2011;).

Pachepsky et al., 2011; Micallef et al.,2012; Park et al., 2013; Ceuppens et al., 2014). Regulations for control of the microbial quality of irrigation water use generic *Escherichia coli* as the indicator organism of the potential human exposure to pathogens. In the United States, the Food Safety Modernization Act (FSMA) (US Congress, 2011) has empowered the U.S. Food and Drug Administration (FDA) to promulgate rules to improve the safety of produce. One of the rules developed by the FDA, the Produce Safety Rule (Federal Register, 2015), specifies regulations for surface irrigation water based on two metrics: the geometric mean (GM) of *E. coli* concentrations and the statistical threshold value (STV) of those concentrations. The GM reflects the central tendency of water quality, and its threshold value is 126 CFU *E. coli* per 100 ml. The STV reflects the variability of the water quality caused by adverse conditions, such as extreme precipitation or high streamflow, and represents the concentration at 90% probability. No more than 10% of samples should exceed the STV threshold, which is 410 CFU *E. coli* per 100 ml (US Congress 2011; Federal Register 2015).

Along with many natural ponds, there are 9 million artificial ponds in the United States (Renwick and Oh 2006), with a large number of them used for irrigation. The microbial quality of water in these ponds, as in other sources of irrigation water, has been mostly unknown (Pachepsky et al., 2011). The concentrations of *E. coli* in ponds are spatially and temporally variable. The statistical distributions of those concentrations are often skewed, with low values found more

often than large ones and standard deviations exceeding mean values (Benjamin et al 2013).

Spatial variability of concentrations creates uncertainty in *E. coli* monitoring results in freshwater sources. Quilliam et al. (2011) demonstrated that microbial water quality on two opposite river banks could suggest very different levels of pollution moving downriver. Jenkins et al. (2012) reported that outflow concentrations of fecal indicator bacteria were significantly lower than inflow concentrations in a pond with perennial flow in Georgia, whereas no such difference was found for ponds with ephemeral flow. If some parts of ponds have *E. coli* concentrations consistently higher than the average *E. coli* concentration across the pond, and other parts of the pond have concentrations that are consistently lower than the average concentration, then a temporally stable pattern of *E. coli* concentrations exists.

Several mathematical methods have been proposed to express temporal stability (Vereecken et al., 2016). Temporally stable patterns across various spatial extents have been observed for various environmental variables, such as soil water content (Vanderlinden et al., 2012), crop yields (Basso et al., 2009), soil nutrients (Anthony et al., 2012), etc., but temporal stability in concentrations of *E. coli* in irrigation ponds has not been studied.

Knowledge of temporally stable patterns appears to be critical for the design and interpretation of environmental monitoring. Microbial water assessment will depend on the choice of sampling locations in the case of well-expressed temporal stability. The objective of this work was to test the hypothesis that the *E. coli* concentrations in irrigation ponds exhibit temporal stability.

Materials and Methods

Pond monitoring

(i) Site description

Two ponds in Maryland were chosen for the current study. These ponds were selected to test the spatiotemporal stability of the microbial indicator organism *Escherichia coli* at approximately the same locations within the ponds throughout the summer of 2016.

(ii) Pond P1

Pond P1, located on a private working farm, is an embankment pond providing irrigation water primarily for the surrounding strawberry fields in the summer (Figure 2.1C). The pond is approximately 91 m long and 68 m in width at its widest points. The average depth is 2.7 m. Small shrubs and deciduous trees grow along the west bank, while other banks are grassed. The topography around the field results in the collection of some runoff from the fields during rainfall events. Runoff can enter the pond from the southwest and north sides, whereas the east side is bordered by constructed fill that diverts water down the backslope and away from the pond. Fields are treated with chemical fertilizers throughout the summer but do not receive animal manures. Irrigation water was drawn intermittently from the pond during prolonged periods of high temperatures at the best judgment of the farm operators. Irrigation never occurred on sampling days. Irrigation was normally applied for 2 to 6 h and did not generate runoff to the pond. Water was pumped from another creek-fed pond into pond P1 occasionally throughout the summer when pond levels were visibly low. Both the inflow and the outflow of the pond are at location

12 in Fig. 2.1C. Pond P1 also served infrequently as a recreational pond, with access on the southwest side.

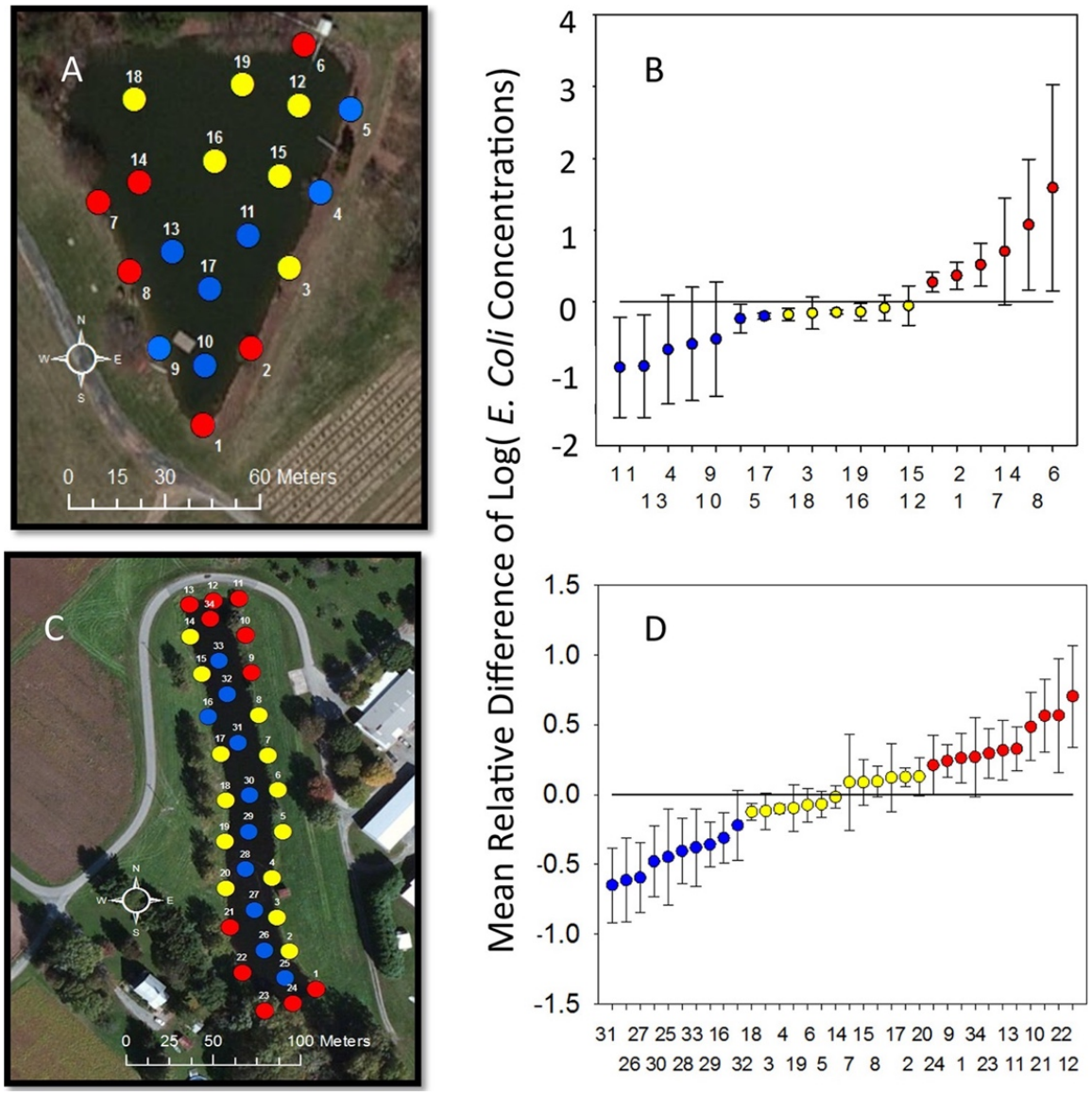

Figure 2.1 Temporal stability of spatial patterns of *E. coli* concentrations in the two ponds studied. (A) Pond P1 with sampling locations. Color coding shows ranking of mean relative differences (MRDs) of logarithms of E. coli concentrations as follows: blue, lowest third; yellow, middle third; red, highest third. (B) MRDs of logarithms of *E. coli* concentrations at sampling locations of pond P1 ordered by rank in ascending order. (C) Pond P2 with sampling locations color coded by MRD rank as described for panel A. (D) MRDs of logarithms of E. coli concentrations at sampling locations of pond P2 ordered by MRD rank. Sampling dates are in Tables 1 and 2. (The images in Panels A and C are from Google Maps [©2017].).

(iii) Pond P2

Pond P2 is an excavated pond located on the University of Maryland Eastern Shore's Wye Research Center. Throughout the observation period, irrigation water was drawn from this pond on nine separate dates at a rate of 155-gal min^{-1} for durations ranging from 1 to 8 h. The irrigation dates were 15 June, 21 June, 21 July, 27 July, 5 August, 8 August, and 10 August and did not coincide with sampling dates except on 10 August, when irrigation water was drawn hours after sampling. The pond is approximately 200 m long and 22 m wide, with an average depth of 2.7 m. The pond is flanked by corn fields on the west side and agricultural supply storage facilities and a parking lot on the east side. The banks of the pond are covered by dense shrubs and grasses with some trees. Pond P2 is at a lower elevation than the surrounding area on the west, north, and east sides but relatively even with the land near the outflow location. The crops around pond P2 receive chemical fertilizers in March, and no animal manures are applied. The water level in the pond is naturally maintained by precipitation, as well as by an ephemeral creek that enters through a culvert at the north end inflow (Figure 2.1A, location 12). This creek routes overland flow from the surrounding corn fields to the pond. The water level in pond P2 is restricted by a water level-dependent orifice outflow drain (Figure 2.1A, location 24) that flows to a ponded marsh-like area that drains into a small creek. The latter transports water away from the system.

Sample collection, handling, and storage

Water samples were collected biweekly from May to September 2016 (Tables 1 and 2). Sampling was conducted on a grid (Fig. 1A and C) at both ponds at a depth from 0 to 15 cm between 9 and 11 a.m. All sampling locations were geotagged using a handheld global positioning system (GPS) device (BE-2300; Bad Elf, Tariffville, CT). Orange flags were placed on the pond exteriors to maintain consistency of bank sampling. Bank samples were collected with field- disinfected (70% ethanol) 500-ml-capacity 6-foot grab samplers and then transferred to sterile Nasco Whirl-Pak bags and placed on ice. Interior pond samples were taken from a kayak. Water samples collected for chlorophyll *a* quantification were kept separately from water samples collected for fecal indicator bacterium enumeration, but both were collected simultaneously with disinfected gear from the same locations and at the same time. The positioning of the interior sampling locations was approximated via reference to bank flags, as well as with the assistance of a land crew. Environmental covariate measurements, including temperature (°C), dissolved oxygen (mg DO liter^{-1}), pH, and conductivity (μS cm^{-1}) were taken in conjunction with water samples using a handheld YSI 556 multiprobe system (MPS; YSI, Inc., Yellow Springs, OH), and turbidity (measured in nephelometric turbidity units [NTU]) was measured in the laboratory (LaMotte Company, Chestertown, MD). Water samples were placed on ice shortly after collection and transported to the laboratory for processing within a couple of hours after collection. Samples remained on ice and in the dark throughout processing. All sampling materials were disinfected with 70% ethanol solution before and after each sampling day.

Laboratory analysis

Membrane filtration was used to enumerate *E. coli*. The filtration volumes varied throughout the experiment based on fluctuations of bacterial concentrations within the sampling period. Sample sizes ranging from 30 ml to 200 ml were filtered through 0.45-µm filters (Millipore Corp., Bedford, MA), which were placed onto modified mTEC (membrane thermotolerant *E. coli*) agar plates (Difco, Sparks, MD). The plates were placed in a 35°C incubator for 2 h and were then transferred to a 44.5°C incubator for 22 to 24 h. After the incubation period, red colonies were counted as *E. coli*. All counts were reported as CFU per 100 ml. Chlorophyll *a* was determined according to the *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association 1995). Nitrate and ammonia concentrations were obtained by flow injection analysis (FIA) on a Lachat QuikChem 8000 series FIA system (Lachat Instruments, Loveland, CO) using Omnion 3.0 software. The QuikChem methodology was modified by using water instead of KCl. Reagents, standards, and manifold settings were prepared according to the QuikChem 12-107-06-2-A and 12-107-04-1-B methods. Orthophosphate concentrations were determined in triplicate according to a modification of the method of Murphy and Riley (1962), using a microplate reader and with the addition of internal standards to each sample.

Temporal stability assessment

The mean relative difference (MRD) (Vachaud et al., 1985) is currently the most common method used to characterize temporal stability. The relative difference

RD_{ij} between the x_{ij}, or observation of variable x at location i at time j, and the $\langle x \rangle_j$, or spatial average of x at the same time, is defined as follows:

$$RD_{ij} = \frac{X_{ij} - (x)_j}{(x)_j}$$

The MRD for the location i becomes

$$MRD_i = \frac{1}{N_t} \sum_{j=1}^{j=N_i} RD_{ij}$$

where N_t is the number of observation times and $i = 1,2,\ldots, N_i$, where N_i is the total number of locations. The standard deviation $SDRD_i$ of the set $RD_{i,1}$, $RD_{i,2}$,. . ., RD_{i,N_t} of relative differences at the location i over the observation period is computed along with MRD_i as follows:

$$SDRD_i = \sqrt{\frac{1}{N_t - 1} \sum_{j=1}^{N_t} (RD_{ij} - MRD_i)^2}$$

This value serves as a metric of the temporal stability for a specific location. The larger the value for $SDRD_i$, the less stable is the mean relative difference MRD_i in the location i.

Observation locations can be sorted by their MRD values. After locations are sorted in the ascending order, i.e., from the smallest MRD to the largest, each location receives a rank which is equal to the position of the location in the sorted MRD array. Location ranking can be used to compare patterns for different variables measured in the same locations. Assuming that locations received ranks R_{xi} based on MRDs for the measured variable X and ranks R^{yi} based on MRDs for the measured variable Y, one can compute the correlation between these two sets of ranks and obtain the

Spearman's correlation coefficient ρ. Values of ρ close to one indicate pattern similarity, whereas values close to -1 indicate the inverse ranking of locations; a large MRD for one of the measured variables corresponds to a small MRD for another variable and vice versa. The probability that the computed value of ρ will be significantly different from zero can be estimated for values of *n* from > 20 observations based on the fact that at a n value of 20, the variable $\rho\sqrt{(n-2)/(1-\rho 2)}$ has an approximately Student's *t* distribution with *n* - 2 degrees of freedom. Microsoft Excel was used in all computations. *E. coli* concentrations, expressed as CFU (100 ml)$^{-1}$, were common log transformed for the statistical analyses.

Results

Spatiotemporal variability of E. coli concentrations and environmental covariates

Statistics of *E. coli* concentrations and environmental covariates are presented in Tables 2.1 and 2.2. No trends of monotonic increase or decrease with time were found for the variables monitored. Temperature, pH, and nitrate concentrations in pond P1 and temperature in pond P2 were found to have low spatial variability, with coefficients of variation (CVs) between 0 and 10%. Nitrate and ammonium concentrations in pond P1 and dissolved oxygen (DO) concentrations, *E. coli* concentrations, turbidity, and orthophosphate concentrations in pond P2 displayed medium temporal stability characterized by coefficients of variation between 10 and 30%. High levels of variation (CVs of >30%) were found for log *E. coli* concentrations and ammonium concentrations in pond P1 and for DO concentrations,

turbidity, and nitrate and ammonium concentrations in pond P2. The concentrations in pond P1 were generally lower than those in pond P2 (see Figure S1 and S2 in the supplementary material). Microbial water quality was found to be satisfactory in each location of both ponds, since the geometric mean concentrations (Figure S2) and the estimated STVs (data not shown) were below the threshold values of 126 CFU (100 ml)$^{-1}$ and 410 CFU (100 ml) $^{-1}$, respectively

Table 2.1 Mean values and standard errors of E. coli concentrations and environmental covariates in pond P1 for 5 sampling dates in 2016

	Avg value ± SE on sampling date				
Parameter	**31-May-16**	**13-Jun-16**	**27-Jun-16**	**11-Jul-16**	**25-Jul-16**
Log (*E. coli* concn) (CFU 100ml^{-1})	0.11±0.38	0.59±0.26	1.36±0.30	1.00±0.30	1.10±0.26
Temperature (°C)	26.56±1.00	24.85±0.39	25.94±0.27	27.15±1.21	26.48±0.53
Conductivity (uS cm^{-1})	154.0±3.73	137.2±0.27	130.0±3.67	136.8±7.68	173.7±0.34
pH	8.56±0.25	8.86±0.21	8.49±0.37	8.24±0.50	9.10±0.57
DO ppm	8.42±0.88	9.17±1.32	9.04±0.83	13.10±1.50	9.94±1.97
Turbidity (NTU[b)]	NC	5.94±0.70	6.58±1.84	8.32±1.88	NC
Nitrate (ppm)	NC	0.87±0.02	0.64±0.01	0.82±0.01	1.19±0.06
Ammonia (ppm)	NC	0.16±0.15	0.01±0.00	0.00±0.02	0.02±0.01
Orthophosphate (ppm)	NC	0.12±0.03	BDL	BDL	BDL

[a]NC, samples not collected: BDL, below detection limit
[b]NTU, nephelometric turbidity unit

Table 2.2 Mean values and standard errors of E. coli concentrations and environmental covariates in pond P2 for 5 sampling dates in 2016

	Avg value ± SE on sampling date					
Parameter	**8-Jun-16**	**22-Jun-16**	**6-Jul-16**	**20-Jul-16**	**4-Aug-16**	**10-Aug-16**
Log (*E. coli* concn) (CFU 100ml^{-1})	1.43±0.25	3.07±0.11	1.01±0.50	0.69±0.75	1.13±0.24	0.77±0.67
Temperature (°C)	23.79±0.71	24.18±0.82	27.43±0.91	28.94±1.67	28.08±0.43	28.12±1.53
Conductivity (uS cm^{-1})	158.4±1.64	172.3±2.46	163.8±0.77	151.3±0.84	155.3±2.25	164.0±1.40
pH	7.99±0.47	6.71±0.51	6.54±0.63	8.19±1.20	7.41±1.44	7.36±1.05
DO ppm	9.19±1.83	5.64±2.72	7.04±3.34	10.16±4.08	6.13±1.26	10.54±3.94
Chlorophyll-*a* (µg liter-1)[b]	NC	137.13±48.19	NC	71.65±7.20	96.2±30.09	320.61±96.15
Turbidity (NTU[c])	27.49±18.04	28.68±21.83	14.92±20.24	72.26±138.35	NC	NC
Orthophosphate (ppm)	NC	0.43±0.05	0.37±0.02	0.21±0.05	0.15±0.02	0.15±0.05

[a]NC, samples not collected: BDL, below detection limit
[b]Measured in the interior of the pond [c]NTU, nephelometric turbidity unit

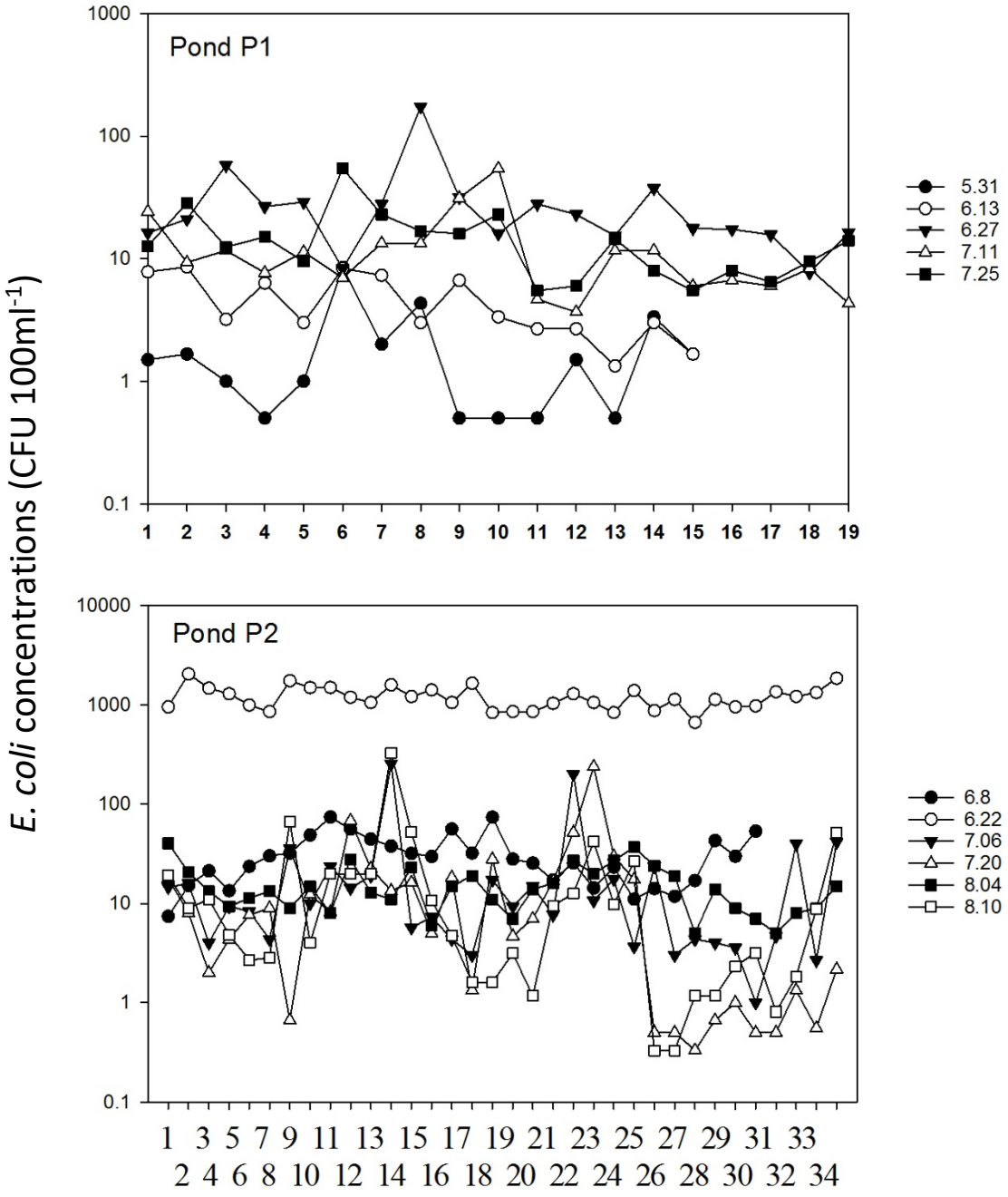

Figure S1 Time series of observed *E. coli* concentrations

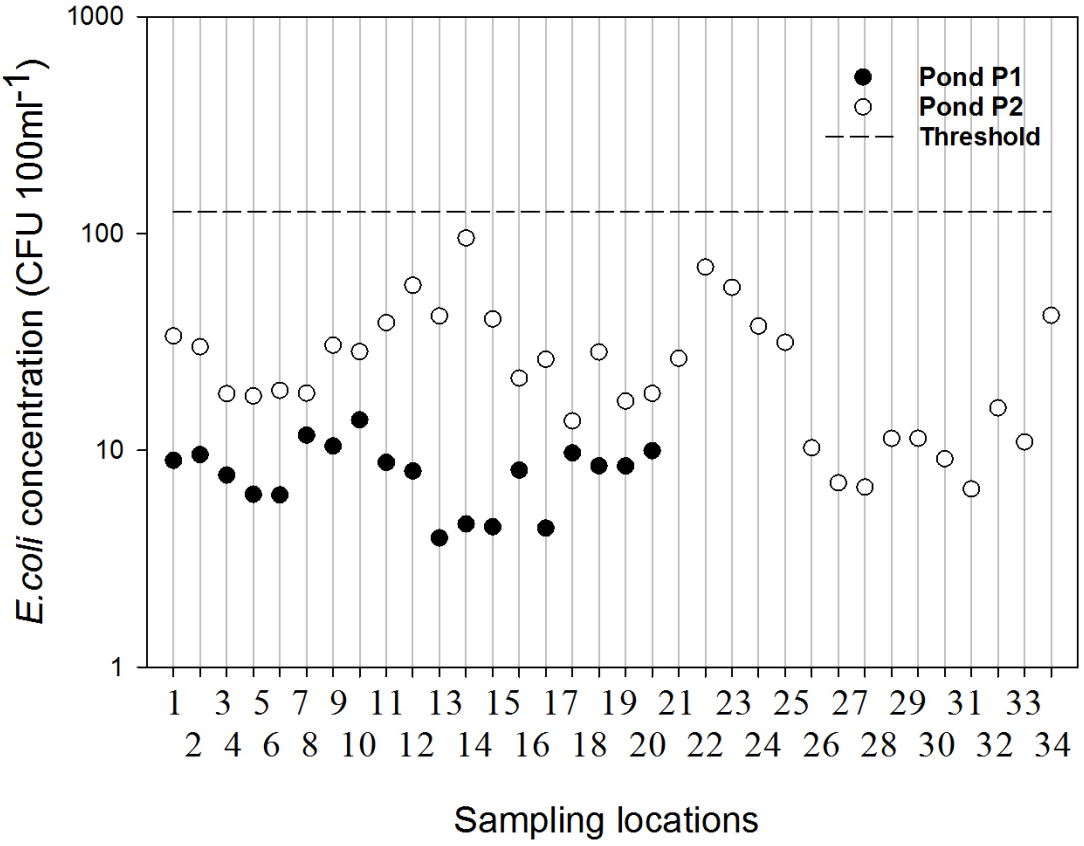

Figure S2 Geometric mean of *E. coli* concentrations by sampling locations at the P1 and P2 ponds relative to the threshold microbial water quality concentration value

Temporal stability of E. coli concentration patterns

The mean relative differences (MRDs) of the logarithms of *E. coli* concentrations for both ponds are shown in Figure 1, with rankings color coded as described in the legend. The temporal stability pattern at pond P1 was well discernible (Figure 2.1A and B). Locations that had high MRDs, i.e., consistently higher than the geometric mean concentrations, were in the recreation area (Figure 2.1A, locations 7, 8, and 14), the direct runoff entrance area (locations 1 and 2), and the inlet-outlet area (location 6). The latter location had the highest MRDs, with *E. coli* concentrations on average 30 times higher than the geometric mean. The interior of the pond was represented by locations that had close to zero or negative MRDs. Locations 11 and 13 had the lowest MRDs, corresponding to *E. coli* concentrations 10 times lower than the geometric mean.

In pond P2, samples close to banks had medium or high MRDs and, therefore, concentrations mostly higher than the median values, whereas concentrations in the samples from the pond interior were mostly lower than the median values (Figure 2.1C and D). The highest MRDs were found in samples taken near the banks close to the pond inlet and outlet. The difference between the highest and the lowest MRD for pond P2 was 1.35. At location 12 near the bank at the pond inlet, the *E. coli* concentrations were on average 5 times higher than the geometric mean concentration. At the same time, the *E. coli* concentrations were on average 5 times lower than the geometric mean concentration at location 31, which is in the pond interior relatively close to location 12. Locations along the banks further from the

inlet and outlet had *E. coli* concentrations that were on average close to the geometric mean (Figure 2.1C and D, color coded in yellow).

Relationships between temporal stabilities of E. coli and environmental covariates

Temporal stability patterns were found for all environmental covariates (Figure S3 and S4). The spread of MRD values, shown in Figure S3 and S4, was much smaller than that for the logarithms of *E. coli* concentrations, shown in Figure S1. The smallest differences were found for temperature, with MRD values varying between 0.04 and 0.05 at pond P2 and between -0.02 and 0.15 at pond P1. For other environmental variables, the spreads were also higher in pond P2 than in pond P1. For example, the DO MRD ranged from -0.4 to 0.4 at pond P2 and from -0.2 to 0.15 at pond P1. Turbidity had the highest spreads.

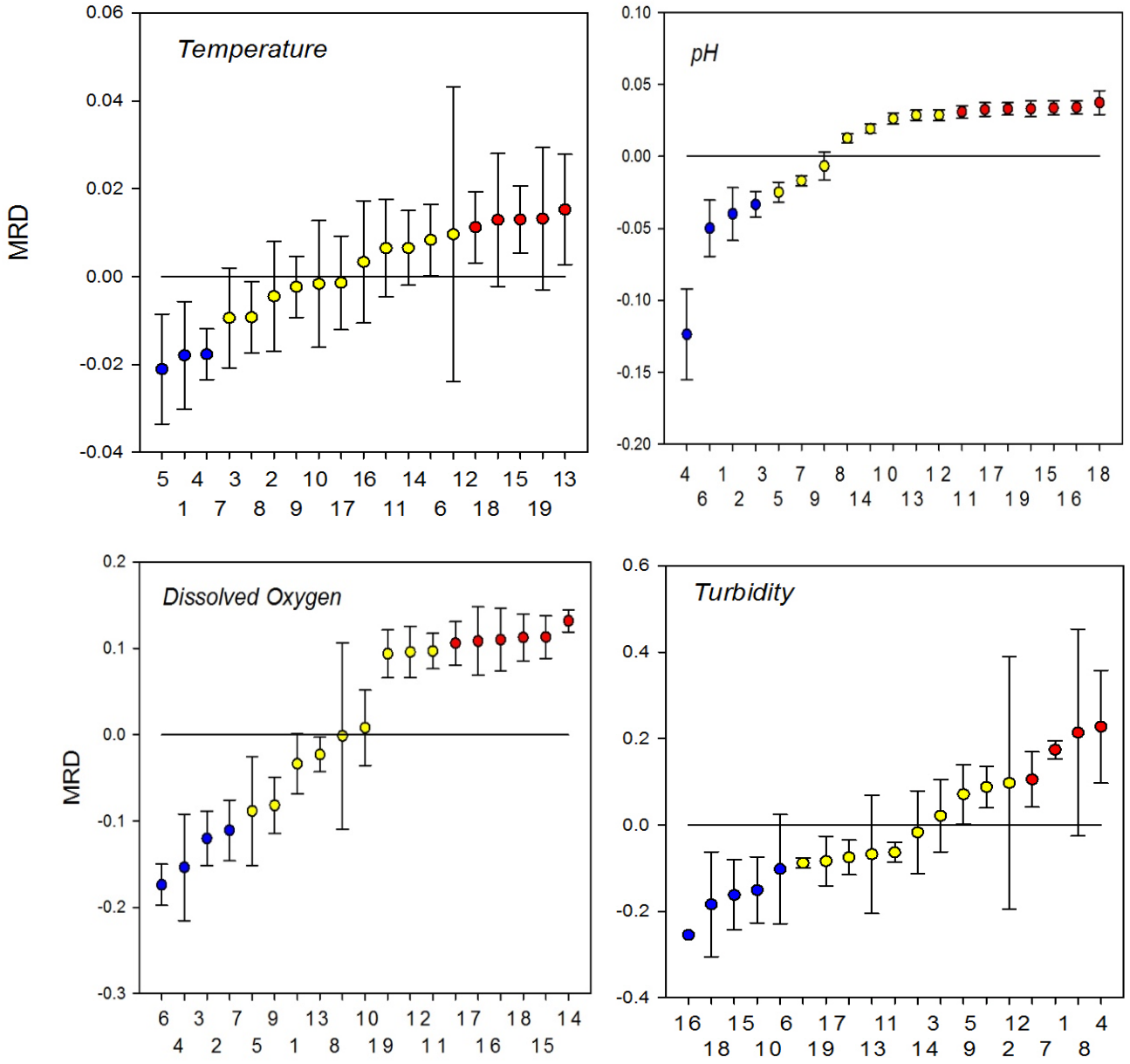

Figure S3 Mean relative differences of selected monitored variables by sampling locations at the P1 pond. Blue- the lowest of the third ranks, yellow- the middles of third, and red- the highest MRD ranks.

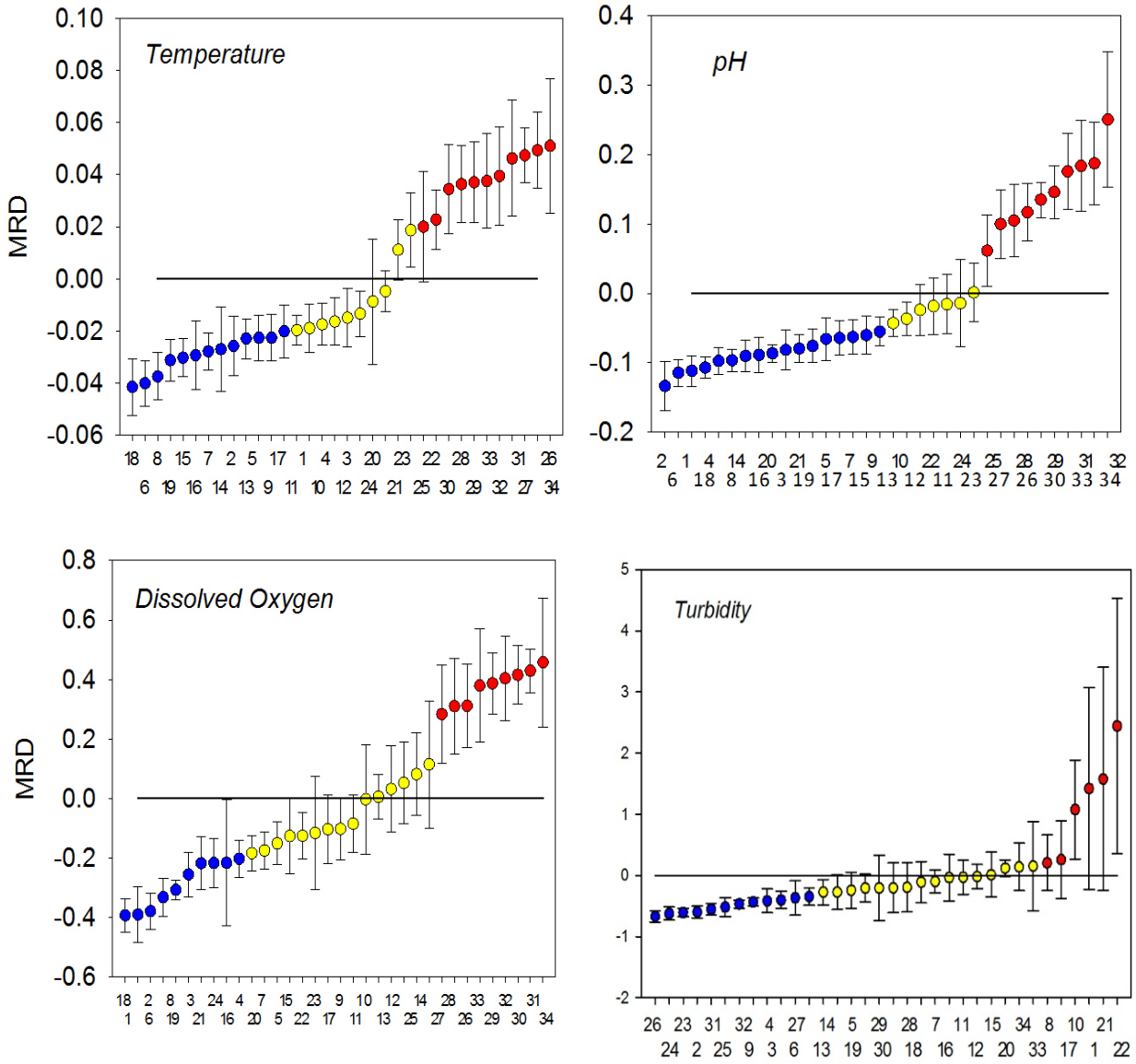

Figure S4 Mean relative differences of selected monitored variables by sampling locations at the P2 pond. Blue- the lowest of the third ranks, yellow- the middles of third, and red- the highest MRD ranks.

The distribution of sampling locations by the MRD rank groups is shown in Figure 2.2. The two last columns for each pond in this figure contain the average ranks of locations close to the banks and in the pond interior. On average, locations close to the banks had slightly higher ranks of log *E. coli* concentration MRDs than the interior locations in both ponds. The difference between the average bank and interior MRD ranks was much higher for other variables observed. Temperature, pH, and DO near the banks had average ranks that were about 1/3 of the average ranks in the interiors, and the opposite was true for turbidity. This means that temperature, pH, and DO were substantially greater near the banks than in the interiors of the ponds, and the turbidity was substantially higher in the pond interiors.

Pond P1

Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	B	I
Log(*E.coli* concentrations)																				10.9	9
Temperature																				5.9	14.6
pH																				5.6	14.9
Dissolved Oxygen																				5.6	14.9
Turbidity																				13.9	5.7

Pond P2

Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Log(*E.coli* concentrations)																					
Temperature																					
pH																					
Dissolved Oxygen																					
Turbidity																					

Location	22	23	24	25	26	27	28	29	30	31	32	33	34	B	I
Log(*E.coli* concentrations)														18.3	15.6
Temperature														12.6	29.2
pH														12.5	29.5
Dissolved Oxygen														12.6	29.2
Turbidity														19.8	13.9

Figure 2.2 Ranking of mean relative differences for observed variables at sampling locations and average ranks near banks (B) and in the interior (I) of ponds in this study. Ranks are color coded as follows: blue, lowest third; yellow, middle third; red, highest third.

Spearman's rank correlation coefficients did not show any strong relationships between the ranks of log *E. coli* concentrations and the ranks of environmental variables (Table 2.3). At pond P1, the MRD ranks of pH and DO were significantly correlated. The turbidity MRD ranks were negatively correlated with the MRD ranks of pH and dissolved oxygen. Conductivity was found to have significant positive correlations with temperature, pH, and DO, but only in pond P1. Conductivity showed a significant negative correlation with turbidity in pond P1. At pond P2, significant positive relationships were found between DO concentrations and both temperature and pH levels. Nitrate concentrations showed a significant but moderate correlation with temperature. The values for pH and temperature were found to have a significant positive relationship.

Temporal stability of chlorophyll a concentrations

Chlorophyll *a* was measured along the transect between locations 25 and 34 in the interior of pond P2. The concentrations ranged from 2.4 to 865.1 μg liter^{-1} over the observation period and exhibited a temporally stable spatial pattern (Figure 2.3a). The chlorophyll *a* MRD ranks increased along the transect in the outlet-to-inlet direction, which means that the amounts of algal and cyanobacterial biomass tended to be larger as the distance to the inlet decreased. The data in Figure 2.3b show a strong negative relationship between the ranks of *E. coli* MRDs and chlorophyll *a* MRDs along the transect, with a Spearman's correlation coefficient of 0.819. Higher *E. coli* concentrations relative to their average across the transect corresponded to smaller chlorophyll *a* concentrations relative to their average across the transect.

Table 2.3 Spearman's correlation coefficients for *E. coli* concentrations and environmental covariates

	Spearman's ρ for indicated covariate [a]						
Variable	**Log(*E.coli concn*)**	**Temperature**	**pH**	**DO**	**Turbidity**	**Nitrate**	**Ammonium**
Log (*E. coli* concn)		**-0.247**	**-0.267**	**-0.246**	**-0.293**	**0.015**	**-0.22**
Temperature	-0.026		**0.765****	**0.764****	**-0.109**	**-0.529***	**-0.253**
pH	0.211	0.686*		**0.919****	**-0.138**	**-0.117**	**-0.213**
DO	0.053	0.528	0.807**		**0.114**	**-0.028**	**-0.268**
Turbidity	0.472	-0.389	-0.602*	-0.660*		**0.096**	**0.423**
Nitrate	-0.256	0.3	0.312	0.196	-0.211		**0.297**
Ammonium	-0.033	-0.018	-0.221	-0.114	0.011	0.377	

[a] Data in lightface are for pond P1, and data in boldface are for pond P2. **, P<0.001: *, P<0.01

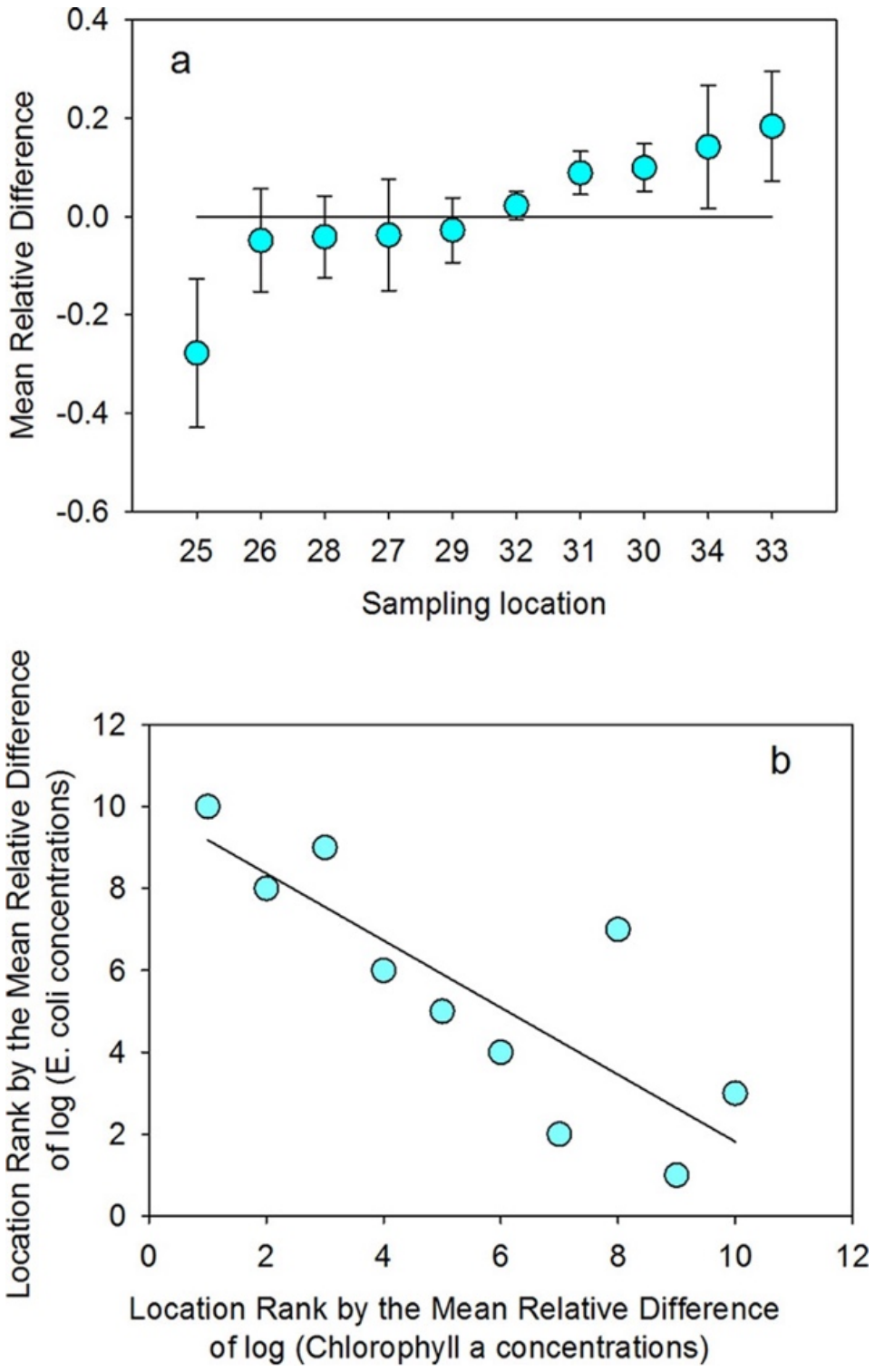

Figure 2.3 Chlorophyll a concentrations in the interior transect of pond P2: temporal stability (a) and relationships between the sampling location ranks by logarithms of concentrations of *E. coli* and chlorophyll a (b).

Concentrations and prevalence of E. coli in P2 pond sediments

On 10 August 2016 and 14 September 2016, sediments were sampled at pond P2 in the same locations where water samples were collected. Bank sediments mainly had a pale-colored coarse sandy texture, while interior sediments were dark and organic, with a muck-like consistency. On 10 August 2016, the *E. coli* concentrations were (1.11 ± 0.26) x 10^3 (average ± standard error) and (6.71± 5.25) x 10^1 CFU 100 g^{-1} for interior and bank samples, respectively. *E. coli* was detected in 90% of interior samples and in 21% of bank samples. For the 14 September 2016 sampling, *E. coli* concentrations were (1.13± 0.36) x 10^3 and (2.38± 0.49) x 10^2 CFU 100 g^{-1} for interior and bank samples, respectively. The prevalence of *E. coli* was 70% for interior samples and 21% for bank samples on the second sampling date. Sediment samples were not collected at pond P1.

Discussion

The *E. coli* concentrations in both ponds studied had temporally stable spatial patterns reflecting differences between sampling locations. In particular, sampling in the inner parts of the ponds provided pathogen concentrations that were consistently lower than the average. Davis et al. (2005) monitored *E. coli* concentrations in a small (1.2 km^2) monomictic reservoir in southeastern California and reported substantially higher concentrations in the shallow eastern area than in the rest of the water body. Jenkins et al. (2012) used tracers to estimate the residence time of microorganisms in Bishop Pond, which had perennial flow through, and found that ideal complete

mixing within Bishop Pond was never obtained. The long residence time meant that fecal bacteria were exposed to solar UV radiation and microbial predation; furthermore, long residence times selected for high algal and cyanobacterial concentrations. At the Bishop Pond outflow location, the concentrations of fecal indicator bacteria were significantly lower than the concentrations at the inflow. The ponds in our work did not have perennial flowthrough. Nevertheless, a concentration gradient along the inlet-outlet transect in the interior of pond P2 (locations 25 to 33) was observed, as evidenced by the sequence of ranks shown in Figure 1B and in Figure S1 in the supplemental material. No bacterial concentration gradient was found at pond P1, where the inlet and outlet concentrations were similar (Figure 1C, location 6).

Recreational activity at the banks could affect the pattern of *E. coli* distribution in pond P1 near locations 6, 8, and 14, which had the highest-ranked concentrations (Fig. 1C and D). Francy et al. (2006) observed that concentrations of *E. coli* were lower in nearshore samples collected 150 ft from the shoreline than in those collected within a swimming area in Lake Erie. Swimming area bed sediments appeared to be important reservoirs of *E. coli* in their system. There are indications that indicator microorganisms can move from sediments to the water column in the absence of substantial resus- pension in streams (Grant et al., 2011; Stocker et al., 2016; Pachepsky et al., 2017), and the same process might affect concentrations in ponds. Differences in sediment composition in different parts of ponds along the banks also may matter. Sediment composition was shown to influence spatial variation in the abundances of human pathogen indicator bacteria within an estuarine

environment (Perkins et al. 2014). In this work, sediments had relatively low levels of *E. coli* compared to the levels found in other, previously observed freshwater systems (Pachepsky and Shelton, 2011), with fine sediments hosting elevated *E. coli* concentrations compared with the concentrations in coarse sediment bank areas.

The highest *E. coli* concentrations were found in locations near the inlets and outlets of the ponds, i.e., locations 12, 13, and 11 and 23, 24, and 1 at pond P2 and location 6 at pond P1 (Figure 1). These locations had also high MRD ranks for turbidity. One can hypothesize that the high turbidity in the absence of flow may be caused by very fine particles or the presence of suspended organic flocs; the latter have been shown to improve the survival of *E. coli* (Droppo et al., 2009). Determining the presence and contribution of such fine-grain, high-surface-area particles and flocs in inflow and outflow zones could be an interesting monitoring component for future work.

The existence of temporal stability of concentrations of *E. coli* can potentially be caused by differences in survival in different parts of the pond. The trees on the banks provide shade. However, recent results indicate that sunlight is not necessarily the dominant factor in *E. coli* survival. Indigenous microbiota and habitat influenced *Escherichia coli* survival more than sunlight in simulated aquatic environments in a study performed by Korajkic et al. (2013). Furthermore, wind is known to be a driver of *E. coli* concentrations at beaches (e.g., see Amorim et al., 2014), providing fine material resus- pension and *E. coli* release (Hutchinson et al., 2008, Ravva et al., 2011). Monitoring wind may, therefore, shed additional light on the observed variations of *E. coli* concentration in ponds. Benjamin et al. (2013) determined that

wind speed and the distance to rangeland were the only environ- mental variables that could serve as predictors of microbial water quality in surface freshwater sources used to irrigate leafy greens in California. Dada and Hamilton (Dada and Hamilton, 2016) reported a correlation between wind speed and *E. coli* concentrations but not with the wind direction at the beaches of a large freshwater lake in New Zealand. These authors suggested that this might be evidence of the lake experiencing wind-driven resuspension of sediments and chronic high turbidity. The role of wind in the formation of spatial patterns of *E. coli* concentrations in ponds has not been studied, and investigations conducted in regions of the world with wind speeds different from those experienced in our region should provide further evidence of whether or not this factor plays a role in produce contamination (Sondergaard et al., 2003; Hickey and Gibbs, 2009).

The standard deviations of the logarithms of *E. coli* concentrations were about 0.3 for pond P1 and from 0.1 to 0.8 for pond P2, which appears to be typical for *E. coli* variation in water bodies. For example, the standard deviation of the log *E. coli* concentration was about 0.6 in pond and reservoir water in central California (Benjamin et al., 2013). In our study, the spatial variation was smaller than the temporal variation (Tables 1 and 2). The latter provided the major proportion of the total variation of microbial water quality, as found in other systems (e.g., see Doering, 1996; Whitman et al., 2004). In the work of Amorim et al. (2014), spatial variation explained about 25% of the total spatiotemporal variability.

Environmental variables also demonstrated patterns of temporal stability (Figure S3 and S4). The ranking of sampling locations by these variables did not have

significant relationships with the ranking of log *E. coli* concentrations, as shown by the data in Table 3. The relationships between ranks of locations by the environmental covariates were similar to the previously observed relationships between these covariates. Beutel and Larson (2015) observed a weak but significant positive correlation between DO and pH and fecal coliform (FC) removal in biofilters, possibly because of the ability of oxygen and hydroxide to enhance sunlight-driven inactivation of pathogens. Addition- ally, during the day, algal photosynthetic activity converts dissolved CO_2 into organic matter and oxygen. This is accompanied by HCO_3 dissociation, increasing the pH (Zang et al., 2011). This occurs to a greater extent when water temperatures are between 20 and 35°C, which appears to be the optimum temperature range for the growth of many cyano- bacteria and chlorophytes (Lurling et al., 2012). The mean daily temperatures across sampling locations throughout the experiment ranged from about 24 to 29°C, which may explain the significant positive correlation of temperature with both pH and DO in the present study. The negative correlation between turbidity and dissolved oxygen could be due to the reduced light penetration, which would limit aquatic photosynthesis and reduce oxygen content (Hall et al., 2015). Positive correlations between DO and pH also explain why turbidity was negatively correlated with pH in our work.

The concentrations of chlorophyll *a* also demonstrated temporal stability. It is possible that the inverse relationship between the MRD ranks of chlorophyll *a* and bacteria is due to the effect of sunlight, which facilitated photosynthesis and impeded the survival of bacteria at the sampling depth of this work. However, this does not explain the rank gradient along the interior transect of locations 24 to 33 at pond P2.

Davis et al. (2005) indicated that some studies have shown a positive correlation between bacteria and chlorophyll *a* in freshwater systems (Weinbauer and Hofle, 1998; Silverman et al., 1983). This occurred, in part, because of the release of dissolved organic carbon and other nutrients back into the water column. Better survival of *E. coli* in waters enriched with organic matter was noted in the review by Blaustein et al. (2013). However, the compounds released are a function of the species of algae and cyanobacteria present, and some may be stimulatory while others could be inhibitory. Reduction of water clarity and effectiveness in inactivating solar radiation was mentioned as another possible reason for the positive effect of algal biomass and chlorophyll *a* concentration on *E. coli* survival (Davies-Colley et al., 2000). Possible interactions of algae and cyanobacteria and *E. coli*, as well as the effect of bacterial attachment to solids on these interactions, present an interesting avenue to explore.

Other methods of temporal stability characterization exist and can be applied to this work and similar endeavors. For example, principal-component analysis can be used to find not a single, but multiple spatial patterns if these exist (Vereecken et al., 2016; Perry and Niemann, 2007). Principal component analysis is focused on absolute rather than relative differences between local observations and the average across observation points.

The existence of temporally stable spatial patterns creates multiple implications for monitoring and management of the microbial quality of freshwater sources. Sampling water from zones with predominantly elevated or predominantly lower pathogen concentrations may create a distorted microbial water quality

assessment. One consequence of the temporal stability in indicator concentrations can be receiving false- negative results with composite samples. Kinzelman et al. (2006) note in their analysis of sampling freshwater swimming sites that these false negatives can be caused by dilution effects that would potentially mask an individual high concentration when combined with those with lower levels.

The existence of zones with consistently different concentrations creates an interesting question of the effect of the water intake location on the microbial quality of water delivered to fields. The microbial quality of irrigation water may change over time as water from different parts of the pond is sent to fields. The existence of three- dimensional patterns needs to be researched over the irrigation periods. Further research on the presence of stable spatial concentration patterns as an interannual phenomenon, the seasonality in those patterns, and their response to specific weather and management conditions may eventually lead to mechanistic interpretation and site-specific explanation of the effects of various microbial sources on the microbial quality of irrigation waters. That eventually can make monitoring of the microbial sources an effective complement to the microbiological monitoring of waters them-selves.

The existence of temporal stability of an environmental variable typically has been used to select a single sampling location that would represent the sampled area as a whole. Finding the representative location for microbial water quality was set as an objective of some microbial water quality research (Jovanovic et al., 2017). An additional objective can be testing the representativeness of a single *E. coli* sample collected from a fixed, routine monitoring station for the overall *E. coli* levels within

an irrigation event. It will be interesting to see whether there is one representative concentration for irrigation water coming from ponds to fields.

Conclusions

Substantial relative differences in *E. coli* concentrations were observed between sampling points, and these differences were fairly stable over time. The pond interiors had persistently lower *E. coli* concentrations than the areas near the bank, even though interior bottom sediments had higher *E. coli* levels than the coarser-grain areas most typical of nearshore banks. Moreover, areas near the banks had their own stable differences. Furthermore, the limited chlorophyll data indicated potential algal and cyanobacterial controls on pathogen densities, and as phytoplankton patchiness is characteristic of many systems, biological controls on *E. coli* levels should also be assessed. Without knowing the temporal stability differences, there is a chance that water samples will have persistently lower or persistently higher concentrations than the average levels sampled across a pond. Hence, the relative contributions of water and associated *E. coli* from bank and interior areas and high versus low-biomass locations for exported irrigation water should be known.

The implications of the temporal stability of *E. coli* concentrations for assessment of water's suitability for irrigation have not been substantial in this work, since most of the concentrations and the geometric mean water quality metric were below the FDA-set thresholds. However, these initial data suggest that temporal and spatial stability could govern exceedances above these levels, and hence, assessments should become routine in future use of pond water for irrigation.

The results of this work show that without a rigorous sampling program, the value of irrigation water source monitoring may be jeopardized. A similar conclusion was made previously for recreational waters, and collecting multiple samples was suggested to improve the estimate of true water quality (Boehm, 2007). The appropriate monitoring design for irrigation ponds appears to be an important research avenue.

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Chapter 3: Analysis of the Spatio-temporal Stability of *Escherichia coli* Concentrations in Two Maryland Irrigation Ponds

Abstract

Accurate measurement and regulation of fecal indicator bacteria concentrations in irrigation ponds is of growing concern for human health. *Escherichia coli* (*E. coli*) is the most common fecal indicator used to asses microbial water quality. The purpose of this study was to evaluate the spatial and temporal variability of *E. coli* concentrations over two sampling years in two Maryland irrigation ponds. Based on results of the first year's study it was found that spatial patterns of *E. coli* concentrations exist. We hypothesized that these patterns would persist during the second sampling year. The same two irrigation ponds in Maryland were sampled biweekly, for two consecutive growing seasons. Environmental covariates—temperature, turbidity, conductivity, pH, dissolved oxygen, chlorophyll *a*, and nutrients—were measured in conjunction with *E. coli* concentrations. Spatial stability was assessed using mean relative differences between measurements in each location and averaged measurements across ponds. Log *E. coli* concentrations were analyzed to assess spatial and temporal variance using Empirical Orthogonal Functions (EOFs). EOFs indicated the spatial variance was widely distributed in both ponds for both years. EOFs indicated definite patterns in the temporal variance of both ponds in both years where the majority of the variance was largely described in the first EOF with less significant contributions in the second EOF.

Introduction

The presence and persistence of fecal bacteria in irrigation ponds is of growing concern for irrigation microbial water quality. In the United States alone, over 9.4 million cases of foodborne illness occur annually with bacterial agents being the second leading cause (Scallan et al., 2011). Regulations for measuring and controlling microbial water quality use *Escherichia coli* as a microbial indicator for the presence of potentially pathogenic fecal bacteria. The U.S. Food and Drug Administration (FDA) specifies fecal indicator bacteria limitations within the Food Safety Modernization Act. The limitations are set based on two metrics: the geometric mean (GM) of *E. coli* concentrations and the statistical threshold value (STV) of those concentrations. The GM reflects the central tendency of water quality, and its threshold value is 126 CFU *E. coli* per 100 ml. The STV reflects the variability of the water quality caused by adverse conditions, such as extreme precipitation or high streamflow, and represents the concentration at 90% probability. No more than 10% of water samples should exceed the STV threshold, which is 410 CFU *E. coli* per 100 ml (US Congress ,2011; Federal Register, 2015).

Surface water is becoming a much more utilized source for irrigation. From 2003 to 2008 the number of US farms using only groundwater for irrigation decreased by 9.2% while the number using surface water increased by 6.3% (Pachepsky et al., 2011). The increased utilization of surface water may result in an increased prevalence of foodborne illness outbreak. Surface waters are highly susceptible to contamination from point sources and/or pollutants that are carried during hydrological events. The highest concentrations of pathogens in surface waters

typically occur after rainfall events (Gerba, 2009). Due to the high risk of contamination from fecal sources, the microbial concentrations in surface waters may be quite variable in surface waters.

Escherichia coli concentrations have been reported as highly variable in freshwater systems (Wu et al., 2011; Reeves et al., 2004; Traister and Anisfeld, 2006; Quilliam et at., 2011). Variations in temperature, hydrological events, and seasonality are some of the numerous contributing factors to the spatial and temporal variations in microbial concentrations. Quilliam et al. (2011) found that the spatial variations of *E. coli* concentrations were very different from one side of the River Conwy, UK to the concentrations measured on the other side of the river. Wu et al (2011) found that based on Spearman Rank Correlations, precipitation was significantly correlated with *E. coli* concentration densities. Microbial concentration fluctuations must be considered carefully when monitoring irrigation ponds to obtained representative values for fecal indicator bacteria (FIB).

In order to distinguish bacterial concentration spatial and temporal stability patterns, there are several mathematical methods have been proposed as being appropriate to use (Vereecken et al., 2016). Spearman Rank Correlations and Mean Relative Difference calculations may be used to find correlations between numerous measured variables. Empirical Orthogonal Functions (EOFs), a close relative of Principal Component Analysis (PCA), have increasing been used in climatology, meteorology, geology, hydrology, and soil science to describe the spatiotemporal variability encountered in these scientific areas (Ogallo, 1989; Fox and Metla, 2005). Often the terminology for EOF and PCA analyses are used almost interchangeably in

the scientific literature. Both EOF and PCA represent types of dimensional analyses to visualize scientific data variance in a manner to more easily see spatial and/or temporal variability patterns. Both analysis methods require an orthogonal structural arrangement within the data. The EOF method is a little more forgiving than PCA analyses as covariance estimations may be used to determine the estimated variance values of missing data points so that orthogonality is observed. The EOF method may also be used in a time series manner to examine sample variability in either a spatial and/or a temporal fashion.

Zeinalzadeh and Rezaei (2017) utilized PCA to compare the environmental discharge effects of several activities, including animal breeding and agriculture, on water quality indicator parameters. The study indicated that PCA could accurately account for temporal and spatial water quality fluctuations from upstream to further downstream.

Microbial water quality is dependent on where and when samples are taken, therefore accurately understanding the spatial and temporal patterns of *Escherichia coli* concentrations will allow for more efficient sampling to monitor compliance of FIB criteria. The objective of this work was to test the hypothesis that the *E. coli* concentrations in irrigation ponds exhibit spatial and temporal stability for the two years sampled.

Methods and Materials

Pond monitoring

(i) Site description

Two ponds in Maryland were chosen for the current study. These ponds were selected to test the spatiotemporal stability of the microbial indicator organism *Escherichia coli* at approximately the same locations within the ponds throughout the summer of 2016 and 2017, respectively.

(ii) Pond P1

Pond P1, located on a private working farm, is an embankment pond providing irrigation water primarily for the surrounding strawberry fields in the summer (Fig. 3.1A). The pond is approximately 91 m long and 68 m in width at its widest points. The average depth is 2.7 m. Small shrubs and deciduous trees grow along the west bank, while other banks are grassed. The topography around the field results in the collection of some runoff from the fields during rainfall events. Runoff can enter the pond from the southwest and north sides, whereas the east side is bordered by constructed fill that diverts water down the backslope and away from the pond. Fields are treated with chemical fertilizers throughout the summer but do not receive animal manures. Irrigation water was drawn intermittently from the pond during prolonged periods of high temperatures at the best judgment of the farm operators. Irrigation did not occur on the scheduled sampling days. Irrigation was normally applied for 2 to 6 h at an application rate that did not generate runoff to the pond. Water was pumped from another creek-fed pond into pond P1 occasionally throughout the summer when pond levels were visibly low. Both the inflow and the outflow of the pond are at location 12 in Fig. 3.1A. Pond P1 also served infrequently as a recreational pond, with access on the southwest side.

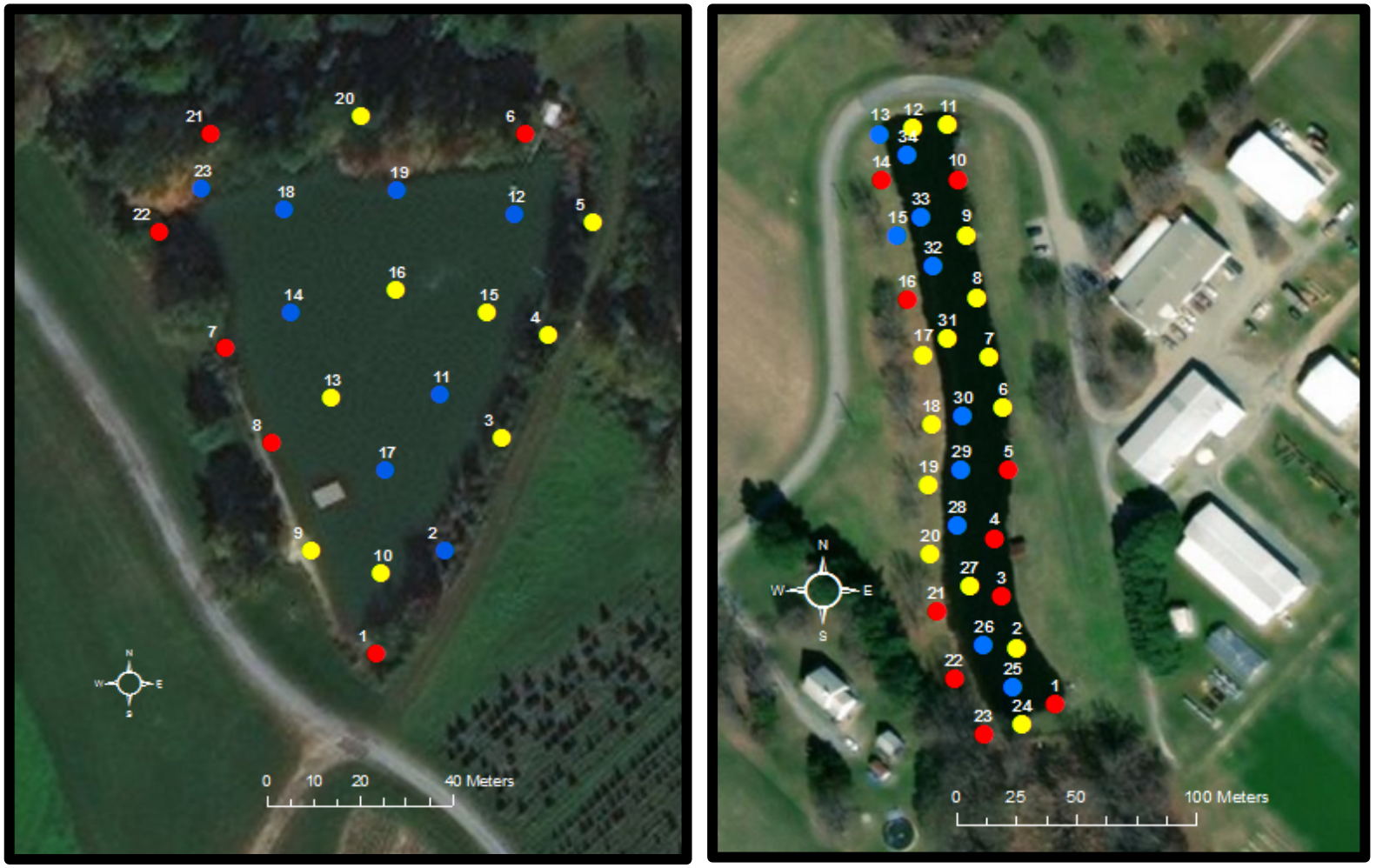


Figure 3.1 Temporal stability of spatial patterns of *E. coli* concentrations in the two ponds studied. (A) Pond P1 with sampling locations. Color coding shows ranking of mean relative differences (MRDs) of logarithms of *E. coli* concentrations as follows: blue, lowest third; yellow, middle third; red, highest third. B) Pond P2 with sampling locations color coded by MRD rank as described for map A. Sampling dates are in Tables 3.1 and 3.2. (The images in Panels A and C are from Google Maps [©2017].).

(iii) Pond P2

Pond P2 is an excavated pond located on the University of Maryland Eastern Shore's Wye Research Center. Throughout the observation period, irrigation water was drawn from this pond on nine separate dates at a rate of 155-gal min^{-1} for durations ranging from 1 to 8 h. The irrigation dates included the 27th of June, 11th of July, and 25th of July which all happen to coincide with sampling days. The pond is approximately 200 m long and 22 m wide, with an average depth of 2.7 m. The pond is flanked by corn fields on the west side and agricultural supply storage facilities and a parking lot on the east side. The banks of the pond are covered by dense shrubs and grasses with some relatively small trees. Pond P2 is at a lower elevation than the surrounding area on the west, north, and east sides but relatively even with the land near the outflow location. The crops around pond P2 receive chemical fertilizers in March, and no animal manures are applied. The water level in the pond is naturally maintained by precipitation, as well as by an ephemeral creek that enters through a culvert at the north end inflow (Figure 3.1B, location 12). This creek routes overland flow from the surrounding corn fields to the pond. The water level in pond P2 is restricted by a water level-dependent orifice outflow drain (Figure 3.1B, location 24) that flows to a ponded marsh-like area that drains into a small creek which transports water away from the system.

Sample collection, handling, and storage

Water samples were collected biweekly from May to September 2016 (Tables 3.1 and 3.2). Sampling was conducted on a grid (Figure 3.1A and B) at both ponds at

a depth from 0 to 15 cm between 9 and 11 a.m. All sampling locations were geotagged using a handheld global positioning system (GPS) device (BE-2300; Bad Elf, Tariffville, CT). Orange flags were placed on the pond exteriors to maintain consistency of bank sampling. Bank and interior samples were collected with 500-ml-capacity 6-foot grab samplers and then transferred to sterile Nasco Whirl-Pak bags and placed on ice. Grab samplers were disinfected with 70% ethanol solution and allowed to dry between each use. Interior pond samples were taken from a kayak. Water samples collected for chlorophyll *a* quantification were kept separately from water samples collected for fecal indicator bacterium enumeration, but both were collected simultaneously with disinfected gear from the same locations and at the same time. The positioning of the interior sampling locations was approximated via reference to bank flags, as well as with the assistance of a land crew. Environmental covariate measurements, including temperature (°C), dissolved oxygen (mg DO liter^{-1}), pH, and conductivity (μS cm^{-1}) were taken in conjunction with water samples using a handheld YSI 556 multiprobe system (MPS; YSI, Inc., Yellow Springs, OH), and turbidity (measured in nephelometric turbidity units [NTU]) was measured in the laboratory (LaMotte Company, Chestertown, MD). Water samples were placed on ice shortly after collection and transported to the laboratory for processing within a couple of hours after collection. Samples remained on ice and in the dark throughout processing. All sampling materials were disinfected with 70% ethanol solution before and after each sampling day.

Laboratory analysis

Membrane filtration was used to enumerate *E. coli*. The filtration volumes varied throughout the experiment based on fluctuations of bacterial concentrations within the sampling period. Sample sizes ranging from 30 ml to 200 ml were filtered through 0.45-µm filters (Millipore Corp., Bedford, MA), which were placed onto modified mTEC (membrane thermotolerant *E. coli*) agar plates (Difco, Sparks, MD). The plates were placed in a 35°C incubator for 2 h and were then transferred to a 44.5°C incubator for 22 to 24 h. After the incubation period, red colonies were counted as *E. coli*. All counts were reported as CFU per 100 ml. Chlorophyll *a* was determined according to the *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, 1995). Nitrate and ammonia concentrations were obtained by flow injection analysis (FIA) on a Lachat QuikChem 8000 series FIA system (Lachat Instruments, Loveland, CO) using Omnion 3.0 software. The QuikChem methodology was modified by using water instead of KCl. Reagents, standards, and manifold settings were prepared according to the QuikChem 12-107-06-2-A and 12-107-04-1-B methods. Orthophosphate concentrations were determined in triplicate according to a modification of the method of Murphy and Riley (1962) method, using a microplate reader and with the addition of internal standards for each sample.

Temporal stability assessment

The mean relative difference (MRD) (Vahaud et al., 1985) is currently the most common method used to characterize microbial temporal stability. The relative

difference RD_{ij} between the x_{ij}, or observation of variable x at location i at time j, and the $\langle x \rangle_j$, or spatial average of x at the same time, is defined as follows:

$$RD_{ij} = \frac{X_{ij} - (x)_j}{(x)_j}$$

The MRD for the location i becomes

$$MRD_i = \frac{1}{N_t} \sum_{j=1}^{j=N_i} RD_{ij}$$

where N_t is the number of observation times and $i = 1,2,\ldots, N_i$, where N_i is the total number of locations. The standard deviation of the relative difference ($SDRD_i$) of the set $RD_{i,1}, RD_{i,2},\ldots, RD_{i,N_t}$ of relative differences at the location i over the observation period is computed along with MRD_i as follows:

$$SDRD_i = \sqrt{\frac{1}{N_t - 1} \sum_{j=1}^{N_t} (RD_{ij} - MRD_i)^2}$$

This value serves as a metric of the temporal stability for a specific location. The larger the value for $SDRD_i$, the less stable is the mean relative difference MRD_i in the location i.

Observation locations can be sorted by their MRD values. After locations are sorted in the ascending order, i.e., from the smallest MRD to the largest, each location receives a rank which is equal to the position of the location in the sorted MRD array. Location ranking can be used to compare patterns for different variables measured in

the same locations. Assuming that locations received ranks R_{xi} based on MRDs for the measured variable *X* and ranks R_{yi} based on MRDs for the measured variable *Y*, one can compute the correlation between these two sets of ranks and obtain the Spearman's correlation coefficient ρ. Values of ρ close to 1.0 indicate pattern similarity, whereas values close to -1.0 indicate the inverse ranking of locations; a large MRD for one of the measured variables corresponds to a small MRD for another variable and vice versa. The probability that the computed value of ρ will be significantly different from zero can be estimated for values of $n \geq 20$ based on the fact that the variable $\rho\sqrt{(n-2)/(1-\rho 2)}$ has an approximate Student's *t* distribution with *n* - 2 degrees of freedom. Microsoft Excel was used in all computations. *E. coli* concentrations, expressed as CFU (100 ml)$^{-1}$, were common log transformed for the statistical analyses.

Another commonly used method to interpret spatial and temporal distributions is Empirical Orthogonal Function (EOF) analysis. The EOF analysis is used to create a set of orthogonal, independent, linear combinations from observations of potentially correlated variables whose interrelatedness may be difficult to interpret (Vereecken et al., 2016). The resulting linear combinations are empirical orthogonal functions and can explain the covariance between the variables (Hartmann 2016). With missing data, Empirical Orthogonal Functions is the preferred method of PCA. The spatial distribution of a variable is the weighted average of the principal components or orthogonal functions dependent on time (Vereecken et al., 2016):

$$X_{i,j} \approx \sum_{k=1}^{K} T_{i,j}\, M_{j,k}$$

"Where X is the original data centered around the spatial average for each observation in time, *i* an index for location, *j* an index for time, *k* an index for EOFs, M_k and the time series of its weights T_k and K the number of such pairs considered." (Vereecken et al. 2016)

This equation may be expanded for temporal and spatial analysis in the following manner where $Y(s,t)$ is the logarithm of *E. coli* concentration in the location *s* at time *t*.

s denotes the sampling location 1, 2, 3 etc.

Spatial EOFs are coefficients of the following expansion

$$Y(s,t) - \bar{Y}(t) = U_1(s)Z_1(t) + U_2(s)Z_2(t) + U_3(s)Z_3(t) + \cdots.$$

Where $\bar{Y}(t)$ is the average logarithm of *E. coli* concentration across all sampling locations at the time *t*.

$Z_1(t),\ Z_2(t),\ Z_3(t),\ Z_4(t),\ Z_5(t),$ ... - are amplitude functions.

$U_1(s), U_2(s), U_3(s), U_4(s), U_5(s),$ … are location-specific coefficients called EOFs.

The EOFs calculated in this manner represent the spatial patterns of sample variance. The first principal component typically explains the most variation in $Y(s,t) - \bar{Y}(t)$, while the second principal component typically explains the second most variation left after the first EOF, etc.

The spatial EOFs explain the variation about the average $\bar{Y}(t)$ over all locations at a specific time. There are EOFs calculated for all sampling locations, but usually only the first few EOF's are statistically significant and characterize the majority of sample population variability. These EOFs are for approximation of variation about the average $\bar{\bar{Y}}(t)$ at all times at a specific location.

Temporal EOFs are coefficients of the expansion defined in the following manner.

$$Y(s,t) - \bar{\bar{Y}}(t) = R_1(t)V_1(s) + R_2(t)V(s) + R_3(t)V_3(s) + \cdots.$$

Where $\bar{\bar{Y}}(s)$ is the average logarithm of *E. coli* concentration over all sampling times at the location s.

$V_1(s)$, $V(s)$, $V_3(s)$ ... - are amplitude functions.

$R_1(t), R_2(t), R_3(t)$, are time-dependent coefficients, or temporal EOFs. The EOFs calculated in this manner represent the temporal patterns of sample variance. The first pattern explains the most variation in $Y(s,t) - \bar{\bar{Y}}(t)$, the second pattern explains the most variation left after the first EOF, etc. (Pachepsky and Hill, 2017)

Spatial and temporal EOFs were calculated on log *E. coli* concentrations from both sampling years, 2016 and 2017. Empirical orthogonal functions (EOF) were determined for the Log (*E. coli*) concentrations using the eof-mca subroutine in R Studio. The EOF analysis was performed in separate analyses for each year emphasizing either the temporal or spatial character of the squared data variance.

Results

Mean values and standard errors of E. coli concentrations and environmental covariates for the two ponds in 2016 and 2017

There were no visible trends of increase or decrease with time for the variables monitored in the 2017 sampling year. *E. coli* concentrations and environmental covariates for 2017 are presented in Tables 3.1 and 3.2. Temperature, pH, conductivity, nitrate and ammonia exhibited the smallest amount of variability for both ponds with standard errors ranging from 0.0 to 0.6 units. The *E. coli* and chlorophyll-a average concentrations were highly variable. In pond P1, log *E. coli* concentrations had a standard error from 0.6-1.7 log CFU/100ml. Pond P2 log *E. coli* concentrations had standard errors ranging from 0.1 to 1.6 log CFU/100ml. Microbial water quality was found to be satisfactory for irrigation use in each location of both ponds, since the geometric mean concentrations (Figure 3.3) and the estimated STVs (data not shown) were below the threshold values of 126 CFU $(100\ ml)^{-1}$ and 410 CFU $(100\ ml)^{-1}$, respectively, which are the required thresholds for using water for irrigation. An exception to this statement occurred for sample location 21 where the *E. coli* concentrations exceeded the threshold levels.

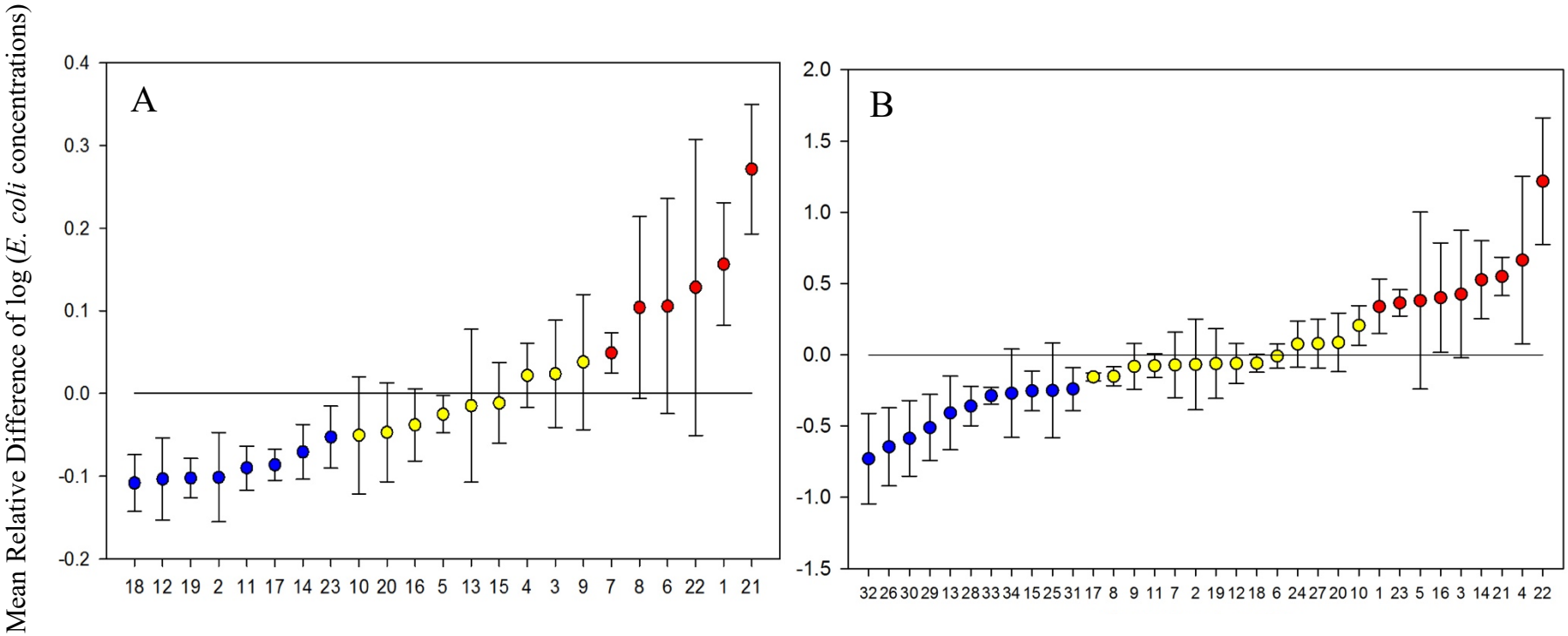


Figure 3.2 Temporal stability of spatial patterns of *E. coli* concentrations in the two ponds studied. (A) MRDs of logarithms of *E. coli* concentrations at sampling locations of pond P1 ordered by rank in ascending order. (B) MRDs of logarithms of *E. coli* concentrations at sampling locations of pond P2 ordered by MRD rank. Color coding shows ranking of mean relative differences (MRDs) of logarithms of *E. coli* concentrations as follows: blue, lowest third; yellow, middle third; red, highest third.

Table 3.1 Mean values and standard errors of *E. coli* concentrations and environmental covariates in pond P1 for the six sampling dates in 2017.

	Avg value ± SE on sampling date					
Parameter	**7-Jun-17**	**21-Jun-17**	**5-Jul-17**	**18-Jul-17**	**1-Aug-17**	**16-Aug-17**
E.coli CFU/100ml	49.0±7.9	82.0±21.8	85.6±47.6	54.8±18.5	70.0±3.8	72.3±12.0
Log (*E. coli* concn)	1.7±0.90	1.9±1.3	1.9±1.7	1.74±1.3	1.8±0.6	1.9±1.1
Temperature C	22.7±0.03	27.1±0.1	28.0±0.06	29.1±0.1	25.4±0.1	25.4±0.1
Conductivity uS/cm	156.4±0.1	168.8±0.2	170.1±0.1	175.8±0.3	150.7±0.1	153.1±0.1
pH	8.5±0.01	8.7±0.1	9.4±0.02	8.9±0.1	8.6±0.1	8.8±0.1
DO ppm	10.5±0.2	9.1±0.2	11.9±0.1	11.0±0.2	10.2±0.1	9.6±0.1
Turbidity NTU[a]	2.9±0.2	4.8±1.0	4.1±0.3	7.4±0.8	6.3±0.1	5.5±0.3
Chlorophyll-a (ug liter^{-1})[b]	4.7±0.58	4.19±0.58	15.8±1.04	15.5±1.06	10.6±0.7	20.2±0.8
Nitrate ppm	0.77±0.005	0.85±0.06	0.6±0.006	0.5±0.01	0.5±0.006	0.3±0.005
Ammonia ppm	0.04±0.004	0.2±0.07	0.04±0.002	0.0	0.0	0.0
Orthophosphate ppm[c]	BDL	BDL	BDL	BDL	BDL	BDL

[a]NTU, nephelometric turbidity unit
[b]Measured in the interior and every odd exterior location number
[c]BDL, below detection limit

Table 3.2 Mean values and standard errors of *E. coli* concentrations and environmental covariates in pond P2 for the six sampling dates in 2017.

	Avg value ± SE on sampling date					
Parameter	**31-May-17**	**13-Jun-17**	**27-Jun-17**	**11-Jul-17**	**25-Jul-17**	**8-Aug-17**
E. coli CFU/100ml	8.2±3.1	9.6±3.0	24.4±5.0	6.9±0.8	16.5±2.8	1177.1±44.0
Log (*E. coli* concn)	0.9±0.5	1.0±0.5	1.4±0.7	0.8±0.1	1.2±0.4	3.1±1.6
Temperature C	24.2±0.21	30.7±0.21	28.0±0.11	29.6±0.12	29.6±0.06	22.2±0.06
Conductivity uS/cm	151.2±0.3	160.2±0.3	169.3±0.24	175.0±0.21	179.2±0.3	141.8±0.5
pH	9.3±0.6	8.72±0.08	8.1±0.1	8.1±0.1	8.1±0.1	6.5±0.0
DO ppm	16.2±0.62	13.8±0.5	13.2±0.68	11.6±0.5	10.1±0.6	5.3±0.06
Turbidity NTU[a]	4.7±0.5	9.6±2.4	6.9±1.2	4.2±0.4	31.8±2.0	5.1±0.2
Chlorophyll-a (ug liter^{-1})	64.3±64.6	10.65±1.96	46.1±4.93	25.6±4.05	170.4±8.04	9.7±0.89
Nitrate ppm	0.04±0.03	0.06±0.03	0.85±0.04	0.05±0.006	0.09±0.01	0.16±0.06
Ammonia ppm	0.05±0.01	0.05±0.005	0.1±0.01	0.02±0.02	0.006±0.006	0.02±0.02
Orthophosphate ppm[b]	BDL	BDL	BDL	BDL	BDL	BDL

[a]NTU, nephelometric turbidity unit
[b]BDL, below detection limit

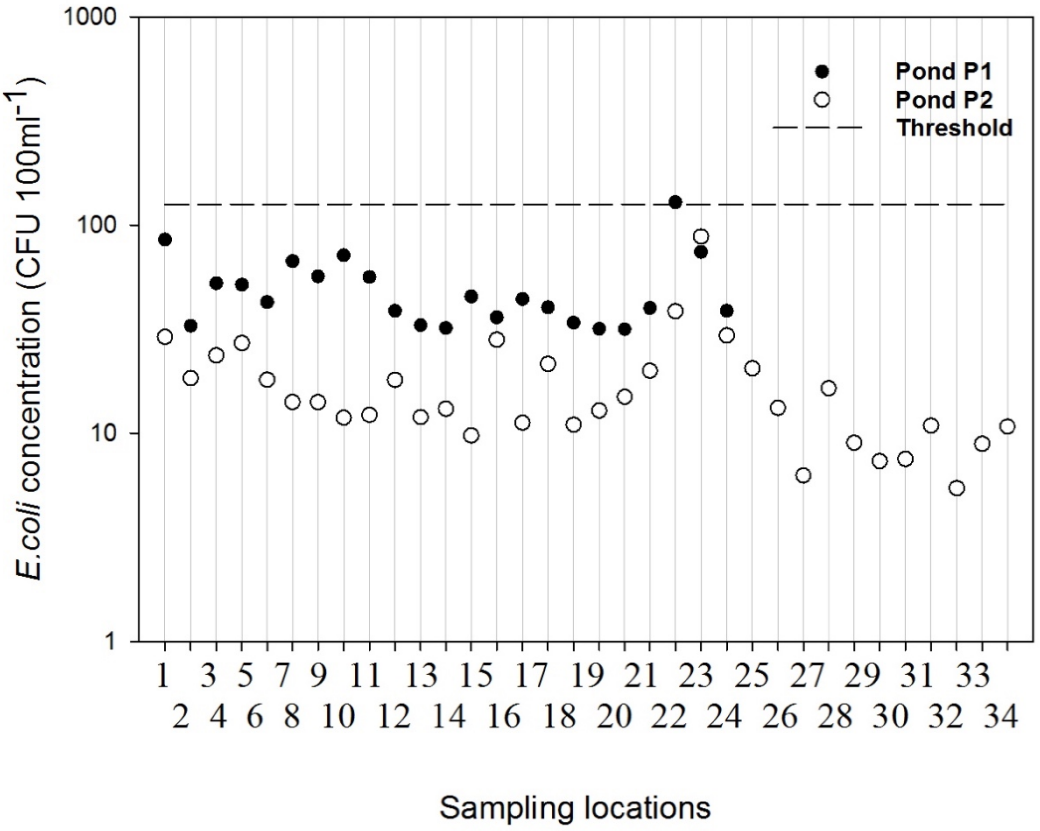


Figure 3.3 Geometric means of the *E. coli* concentrations calculated for each sampling location in both ponds over the sampling period. The dashed line represents the geometric mean criterion limitation according to the FDA FSMA (126 CFU $100ml^{-1}$). The statistical threshold value, 410 CFU $100ml^{-1}$ is not shown.

Temporal stability of E. coli concentration patterns

The temporal stability patterns were discernible for both ponds (Figure 3.2). The mean relative differences (MRDs) of the *E. coli* concentrations for both ponds are shown in Figure 3.4, with rankings color coded as described in the legend. Mean relative difference units for pond P1 ranged from 0.11 units below the mean and 0.27 units above the mean. In pond P2 the MDRs ranged from 0.73 units below the mean and 1.22 units above the mean. The lowest MRD values for pond P1 were at locations 18 and 12, and the highest were at locations 1 and 21. High MRD values correspond with the locations for the pipe intake of the irrigation pump and the recreational beach area as well as the input area from a small ephemeral stream (locations 21 and 22). The highest MRDs for pond P2 were located at locations 4 and 22 and the lowest were at locations 32 and 26. The lowest MRDs were located in both pond interiors. The highest MRD in pond P2 correspond with the runoff entrance area and the outflow.

Relationships between temporal stabilities of E. coli and environmental covariates

Temporal stability patterns were found for all environmental covariates (Table 3.3). The range of MRD values for the covariates were much smaller than the range of MRD values for the log *E. coli* concentrations (Figure 3.4). The smallest differences were found for conductivity, with MRD values varying from -0.007 to 0.005 for pond P1 and -0.06 to 0.015 for pond P2, and temperature, -0.015 to 0.015 for pond P1 and -0.04 to 0.05 for pond P2. The ranges for environmental covariates were higher in pond P2 than pond P1. For example, DO MRD values for P1 ranged

from -0.07 to 0.19 and from -0.18 to 0.3 at P2. These values are consistent with 2016 sampling trends. Turbidity had the largest range of values for both ponds.

Table 3.3 Spearman's correlation coefficients for *E. coli* concentrations and environmental covariates

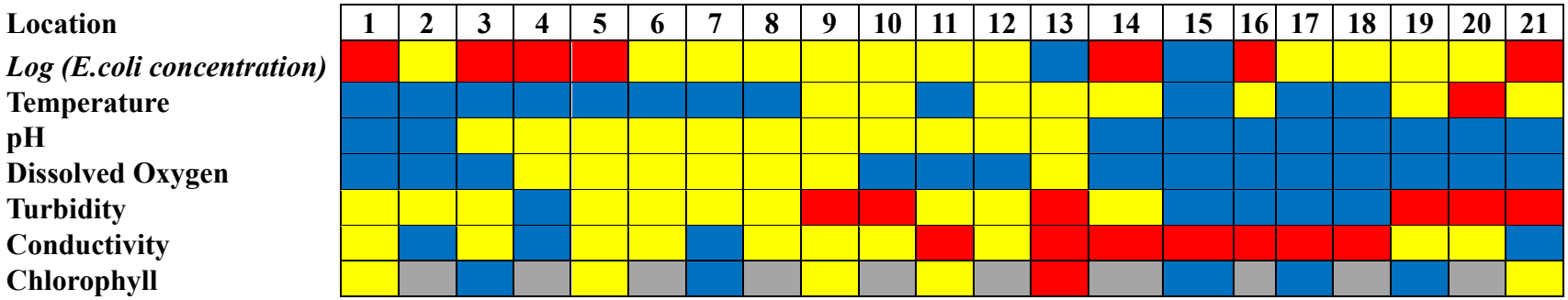
Variable	Spearman's *p* for indicated covariate [a]						
	Log(*E.coli concn*)	**Temperature**	**pH**	**DO**	**Turbidity**	**Conductivity**	**Chlorophyll-a**
Log(*E. coli* concn)		**-0.449***	**-0.584****	**-0.517***	**0.113**	**0.0261**	**-0.301**
Temperature	-0.539*		**0.672****	**0.744****	**0.0215**	**-0.166**	**0.493**
pH	-0.156	0.213		**0.939****	**-0.0588**	**-0.383**	**0.423**
DO	-0.0791	0.242	0.823**		**-0.0487**	**-0.389**	**0.38**
Turbidity	0.531*	-0.52	0.277	0.155		**0.0536**	**0.136**
Conductivity	-0.462	0.53*	-0.0524	-0.111	-0.375		**0.149**
Chorophyll-a	0.308	-0.342	-0.0998	-0.309	0.508	-0.526	

[a] Data in lightface are for pond P1, and data in boldface are for pond P2. **, P<0.001: *, P<0.01

Pond P1

Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	B	I
Log (*E. coli* concentration)																								16.4	7.2
Temperature																								7.0	17.5
pH																								11.3	12.8
Dissolved Oxygen																								11.3	12.8
Turbidity																								16.5	7.1
Conductivity																								8.8	15.5
Chlorophyll																								15.0	8.7

Pond P2



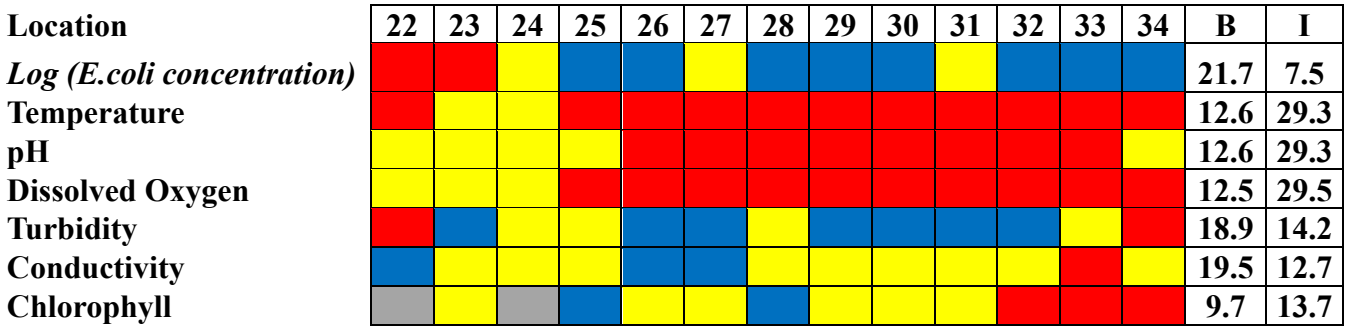


Figure 3.4 Ranking of mean relative differences for observed variables at sampling locations and average ranks near banks (B) and in the interior (I) of ponds in this study. Ranks are color coded as follows: blue, lowest third; yellow, middle third; red, highest third.

The distribution of sampling locations by the MRD rank groups is shown in Figure 3.4. The two last columns for each pond in this figure contain the average ranks of locations close to the banks and in the pond interior. On average, locations close to the banks had higher ranks of log *E. coli* concentrations than the MRDs for the interior locations within both ponds. The differences of average MRD ranks in the interior and banks were very small for pH and DO in pond P1. Temperature, pH and DO for pond P2 and chlorophyll for pond P1 had higher average ranks in the interior than the banks which was opposite for the chlorophyll and turbidity values for pond P1. These values show that the temperature, pH, and DO values were substantially greater near the banks within pond P2 and the chlorophyll and turbidity values were greater in the interior within pond P1.

There were significant Spearman rank correlation coefficients for the log *E. coli* concentrations with the temperature and turbidity values within pond P1 and with the temperature, pH and DO values within pond P2. In pond P1, the DO was positively correlated with pH and conductivity was positively correlated with temperature. Significant positive correlations between temperature and pH/DO and between pH and DO were found within pond P2.

Temporal stability of chlorophyll a concentrations

Chlorophyll *a* was measured at every odd numbered sampling location around the pond P2 banks and in the transect between locations 25-34. In pond P1, the chlorophyll *a* was measured at every sampling location. Chlorophyll *a* concentrations were larger in both pond's interior sampling locations. There were positive Spearman rank correlation coefficients between the chlorophyll *a* and temperature/pH within

pond 2 with p values less than 0.05 suggesting that the pairs of variables tended to increase together. Within pond P1, the chlorophyll values had a positive Spearman rank correlation coefficient with turbidity ($p<0.05$). There was a negative correlation between conductivity and chlorophyll *a* with a coefficient of -0.526 ($p<0.05$) within Pond P1. However, with an alpha of 0.01 these trends were not significant. There was no significant relationship found in the MRD values between chlorophyll *a* and log *E. coli* found within either pond.

Spatial EOFs of log E. coli concentrations

Spatial EOFs of log *E. coli* concentrations for both ponds and sampling years were analyzed separately. The first six spatial EOFS for pond P1and pond P2 2016 and 2017 data are presented in Table 3.4. For pond P1 2016 and 2017 log *E. coli* data, the first two EOFs accounted for approximately 65% of the total variance (Table 3.4). For pond P2 2016 and 2017 data the first two EOFs accounted for approximately 80% of the total variance (Table 3.4). The sampling location first and second EOFs for pond P1 and P2 are displayed in Figures 3.5 and 3.6. There were no significant height differences in the bar graphs between EOF 1 and EOF 2 for either pond or sampling year. Interior sampling locations (blue bars) contributed more variation to the total spatial variation then the bank sampling locations (yellow bars), this is evident by the large positive values of the blue bars.

The first two spatial EOFs values as the product of amplitude functions, U(s) and location-specific coefficient or EOF, Z(t), were plotted against the original data centered about the spatial average ,$(Y(s,t) - \bar{\bar{Y}}(t))$, for both ponds and sampling years in Figures 3.7 and 3.8. For an EOF that captures much of the variance in the

data, this plot should show the data points falling close to the 1:1 ratio line according to the spatial EOF expansion equation (Packepsky and Hill, 2017). Deviations from the 1:1 line indicate times where the EOF analysis is not capturing the maximum amount of variance. The pond P1 data points for 2016 and 2017 did not fit the 1:1 ratio line displayed on the graphs (Figure 3.7). The pond P2 data points did seem to fit the 1:1 ratio line more appropriately (Figure 3.8).

Table 3.4 First six EOF spatial values as percentages for both ponds and sampling years.

Pond P1	EOF 1	EOF 2	EOF 3	EOF 4	EOF 5	EOF 6
2016	38.1	27.2	22.3	8.73	3.60	N/A
2017	39.0	26.0	20.0	10.6	3.00	1.40
Pond P2	EOF 1	EOF 2	EOF 3	EOF 4	EOF 5	EOF 6
2016	61.4	18.5	12.2	5.6	1.70	0.55
2017	50.1	26.0	13.3	7.00	2.80	0.81

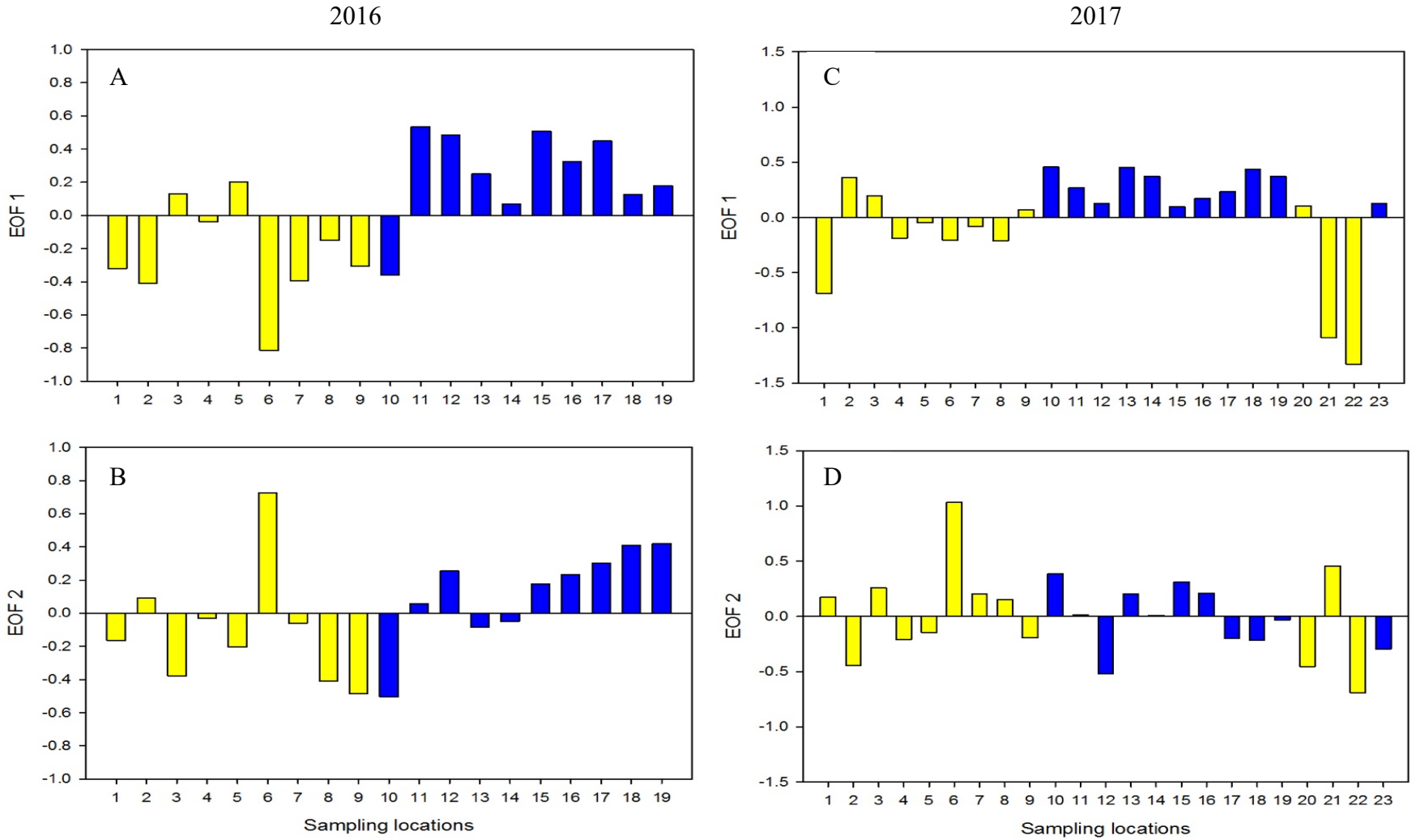


Figure 3.5 Spatial EOFs of log *E. coli* concentrations for pond P1. (A and B) The first and second spatial EOF for 2016. (C and D) The first and second spatial EOF for 2017. The bars are color coded for location: yellow, bank samples; blue, interior samples.

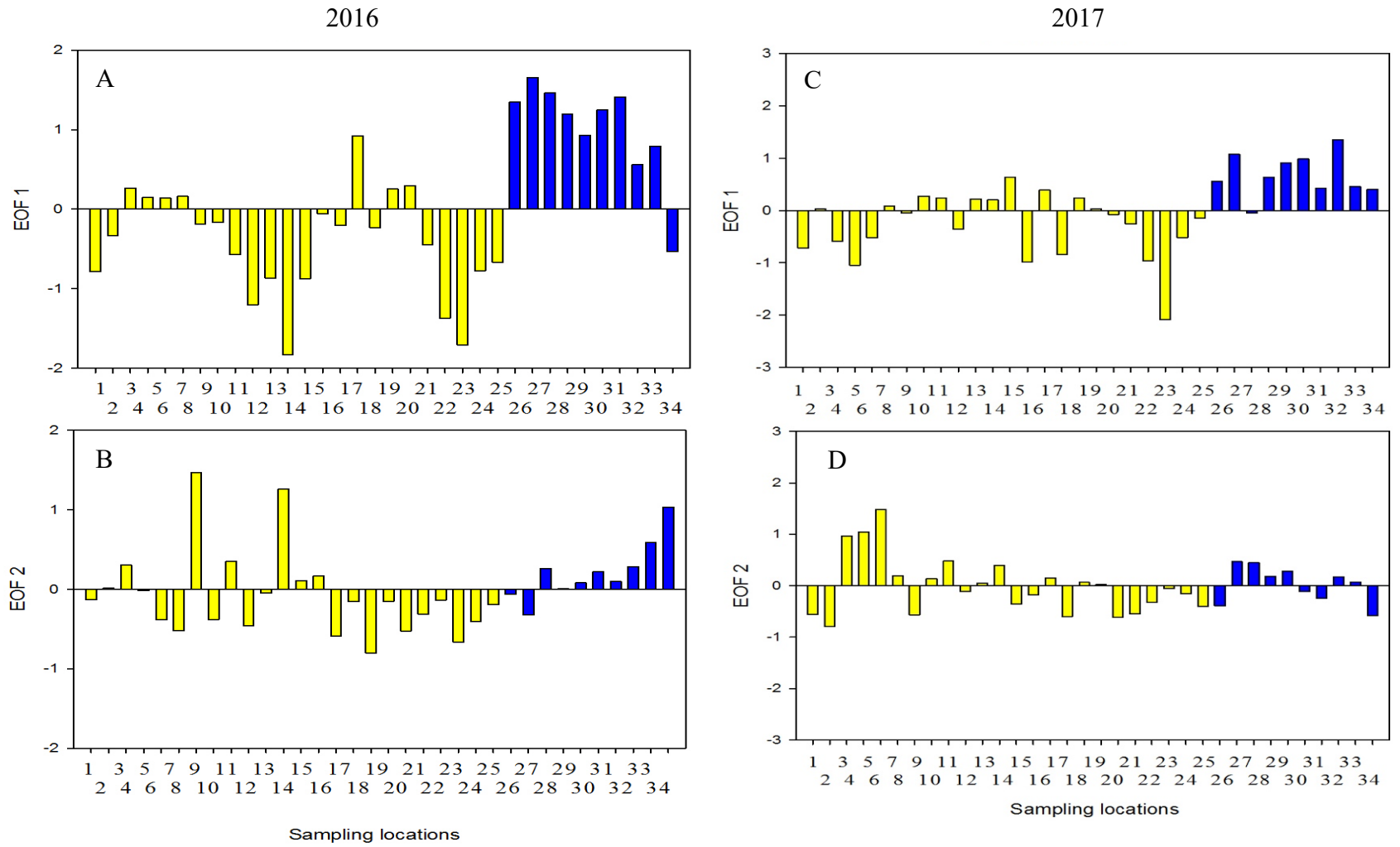


Figure 3.6 Spatial EOFs of log *E. coli* concentrations for pond P2. (A and B) The first and second spatial EOF for 2016. (C and D) The first and second spatial EOF for 2017. The bars are color coded for location: yellow, bank samples; blue, interior samples

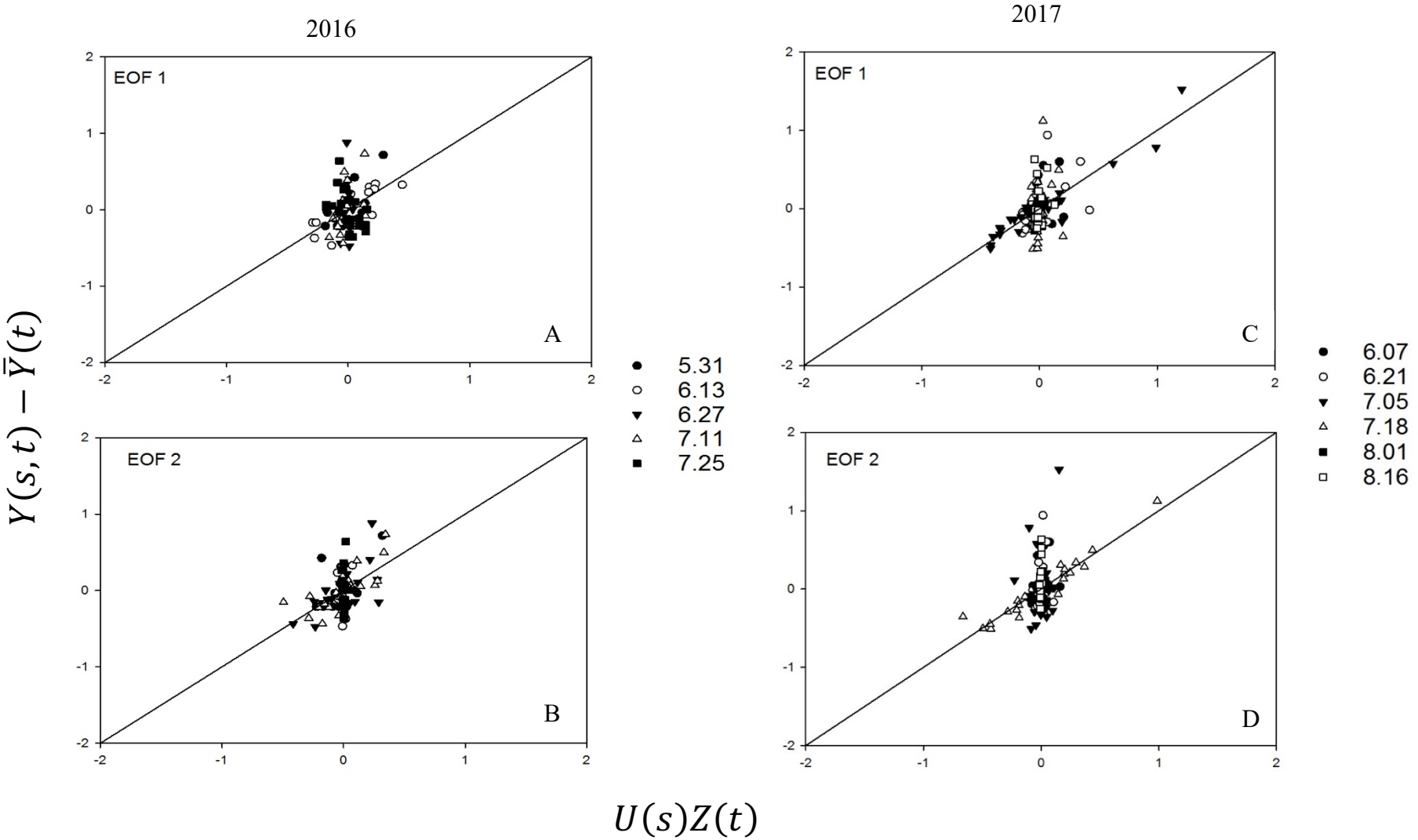


Figure 3.7 First two spatial EOFs of log *E. coli* concentrations for pond P1. (A and B) Spatial EOFs for 2016 separated by sampling date on the right. (C and D) Spatial EOFs for 2017 separated by sampling date on the right.

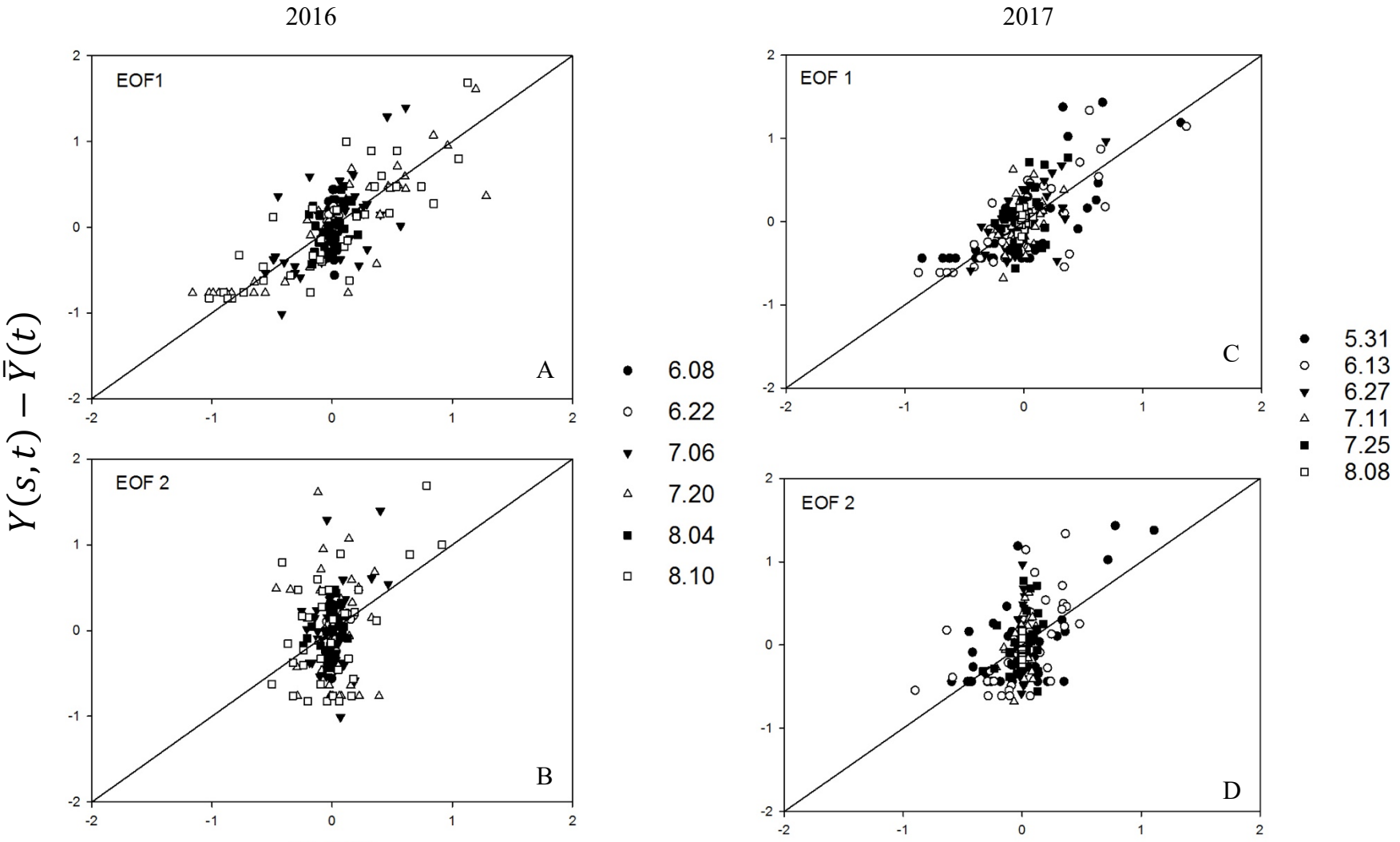


Figure 3.8 First two spatial EOFs of log *E. coli* concentrations for pond P2. (A and B) Spatial EOFs for 2016 separated by sampling date on the right. (C and D) Spatial EOFs for 2017 separated by sampling date on the right.

Temporal EOFs of log E. coli concentrations

Temporal EOFs of log *E. coli* concentrations for both ponds and sampling years were analyzed separately. The first six spatial EOF contribution to the total variation for pond P1 and P2 2016 and 2017 data are presented in Table 3.5. For pond P1 2016, the first two EOFs accounted for approximately 90% of the total variance (Table 3.5). For 2017, the first two EOFS for pond P1 accounted for approximately 73% of the total variance. For Pond P2 the first two EOFs accounted for approximately 90% and 95% of the total variance in 2016 and 2017, respectively. The first and second temporal EOF of each sampling day for pond P1 and P2 are displayed in Figures 3.9 and 3.10. The height of the bar graph for the first EOF, ex: sampling days 1 and 3, for Pond P1 in 2016 (Figure 3.9A) is larger than those for the second EOF for the same year and pond (Figure 3.9B). The graph of the 2017 first EOF for pond P1 (Figure 3.9C) had similar bar heights to the second EOF (Figure 3.9D), ex: sampling date 3. For both sampling years for pond P1 the first EOF bar graph heights (Figure 3.10A and C) were much larger than the second EOF bar graph heights (Figure 3.10B and D).

The first two temporal EOF values as the product of amplitude functions and time-specific coefficients, $(R(t)V(s))$: x-axis, were plotted against the temporal *E. coli* concentrations centered about the temporal average, $(Y(s,t) - \bar{\bar{Y}}(t)$, for both ponds and sampling years in Figures 3.11 and 3.12. The data points for the first EOF in 2016 for pond P1 (Figure 3.11A) follow the 1:1 ratio line. The data points for 2017 for pond P1 (Figure 3.11C) for the first EOF cluster around the 1:1 ratio line and are less conclusive than the 2016 data. The first two temporal EOF data points for 2016

and 2017 for pond P2 exhibit vertical clumping for sampling days 6/08, 6/27, and 7/25. This indicated that on those dates the EOF and amplitude products appear to be constant or nearly so.

Table 3.4 First six EOF temporal values as percentages for both ponds and sampling years.

Pond P1	EOF 1	EOF 2	EOF 3	EOF 4	EOF 5	EOF 6
2016	78.3	11.7	5.50	2.90	1.20	0.33
2017	39.8	32.8	17.5	5.30	4.70	<0.01
Pond P2	EOF 1	EOF 2	EOF 3	EOF 4	EOF 5	EOF 6
2016	86.0	4.80	3.90	2.70	1.30	0.12
2017	91.0	4.30	2.50	1.70	0.70	<0.01

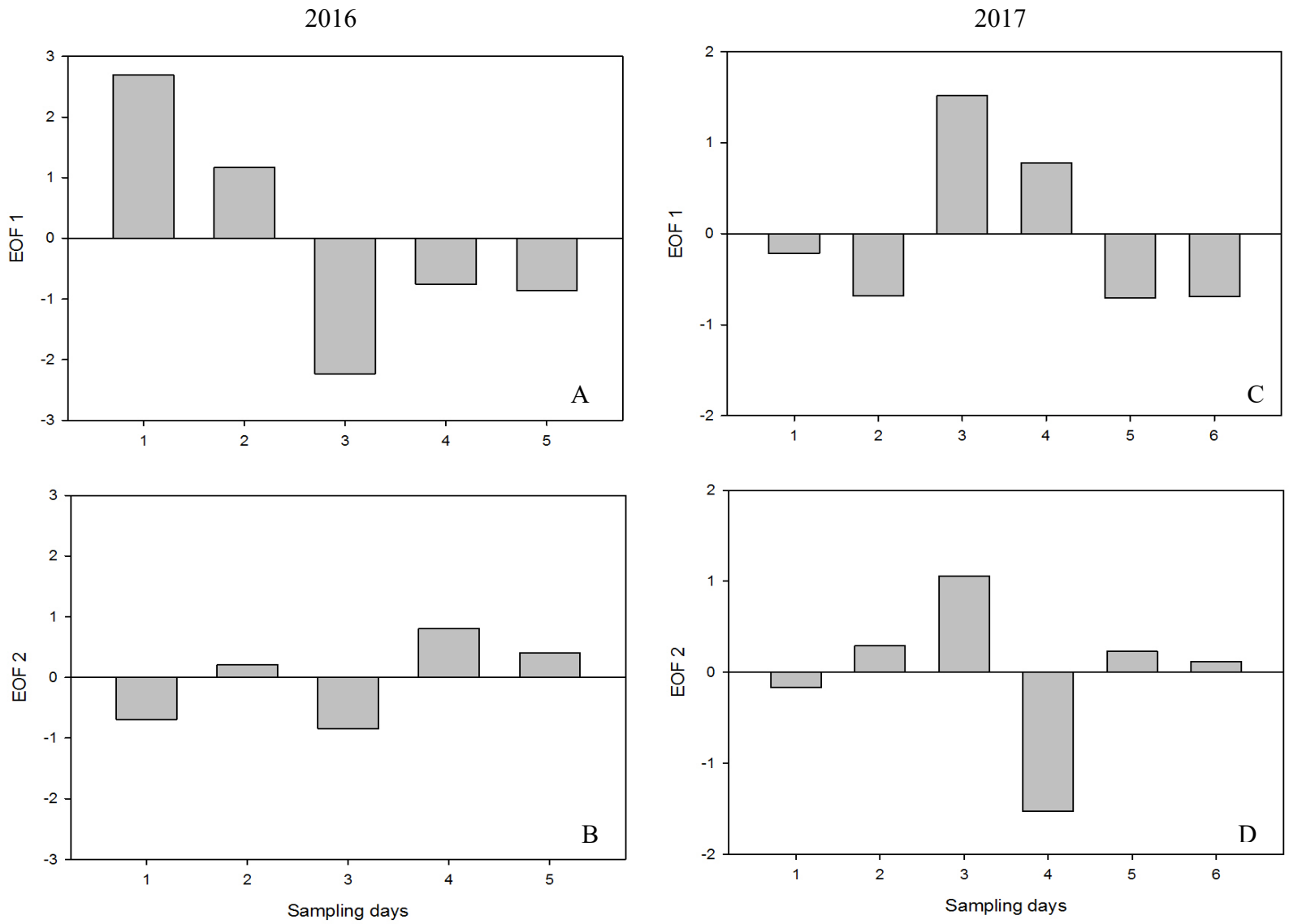


Figure 3.9 Temporal EOFs of log *E. coli* concentrations for pond P1. (A and B) The first and second temporal EOF for 2016. (C and D) The first and second temporal EOF for 2017. The sampling days are numbered on the x axis.

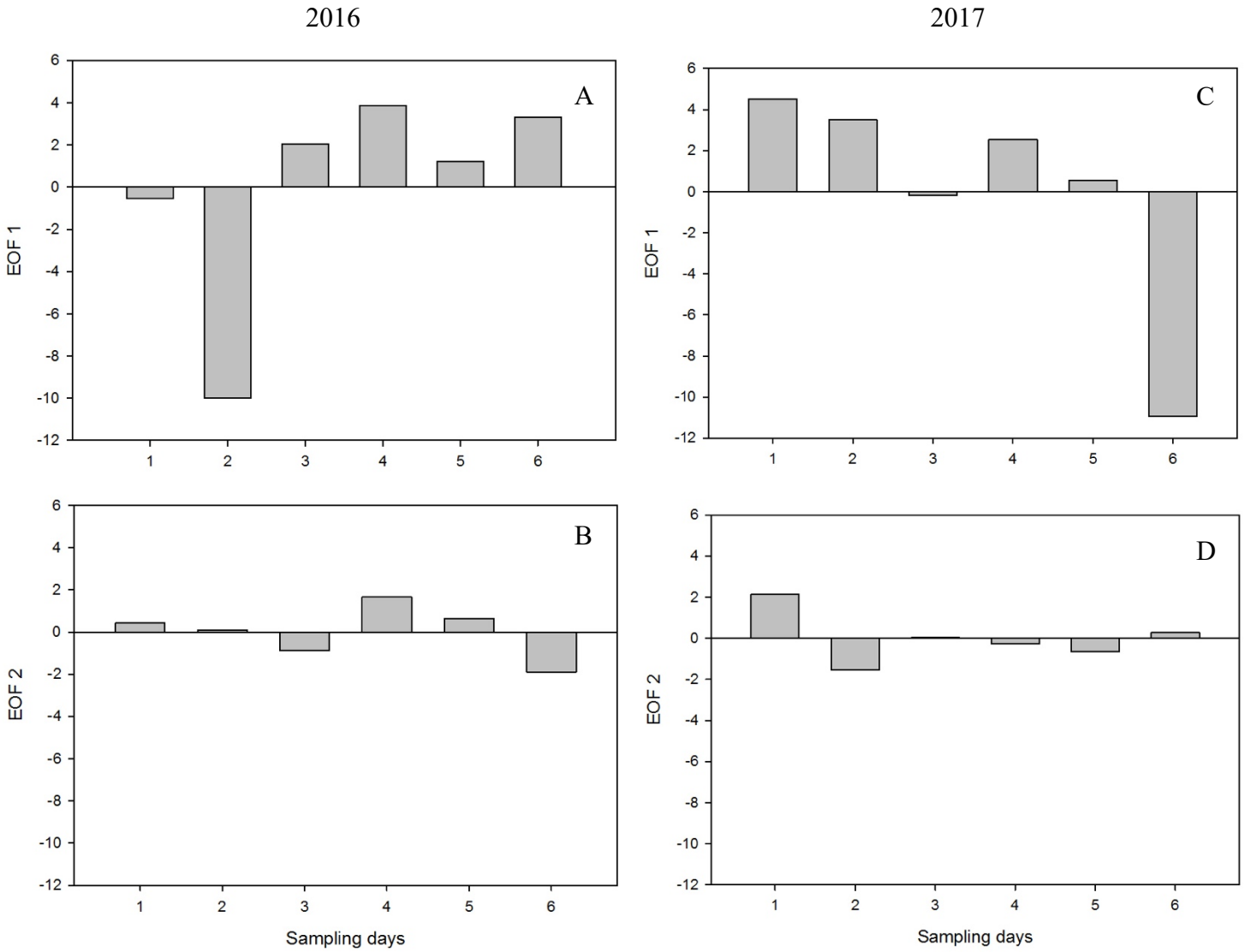


Figure 3.10 Temporal EOFs of log *E. coli* concentrations for pond P2. (A and B) The first and second temporal EOF for 2016. (C and D) The first and second temporal EOF for 2017. The sampling days are numbered on the x axis.

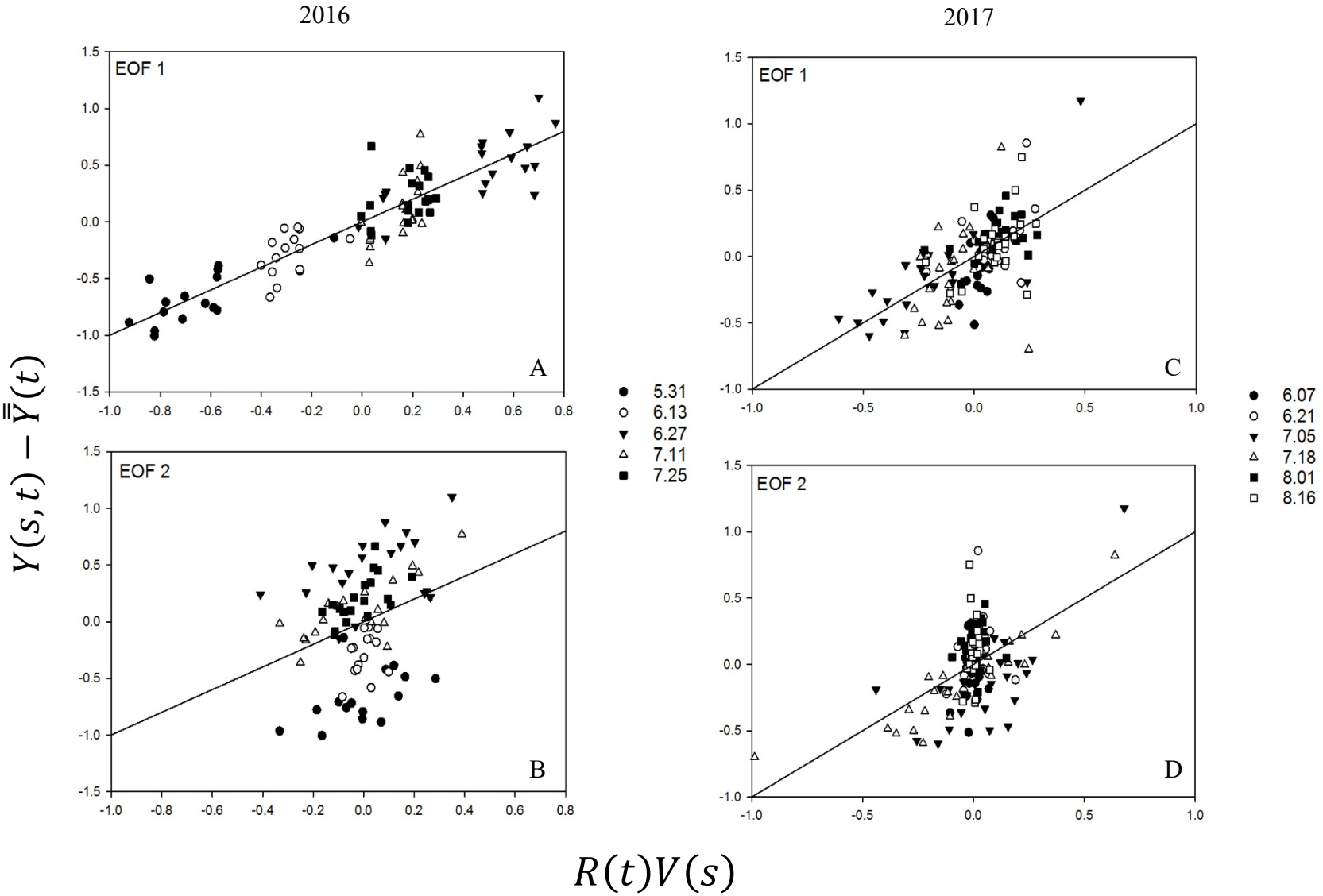


Figure 3.11 First two temporal EOFs of log *E. coli* concentrations for pond P1. (A and B) Temporal EOFs for 2016 separated by sampling date on the right. (C and D) Temporal EOFs for 2017 separated by sampling date on the right.

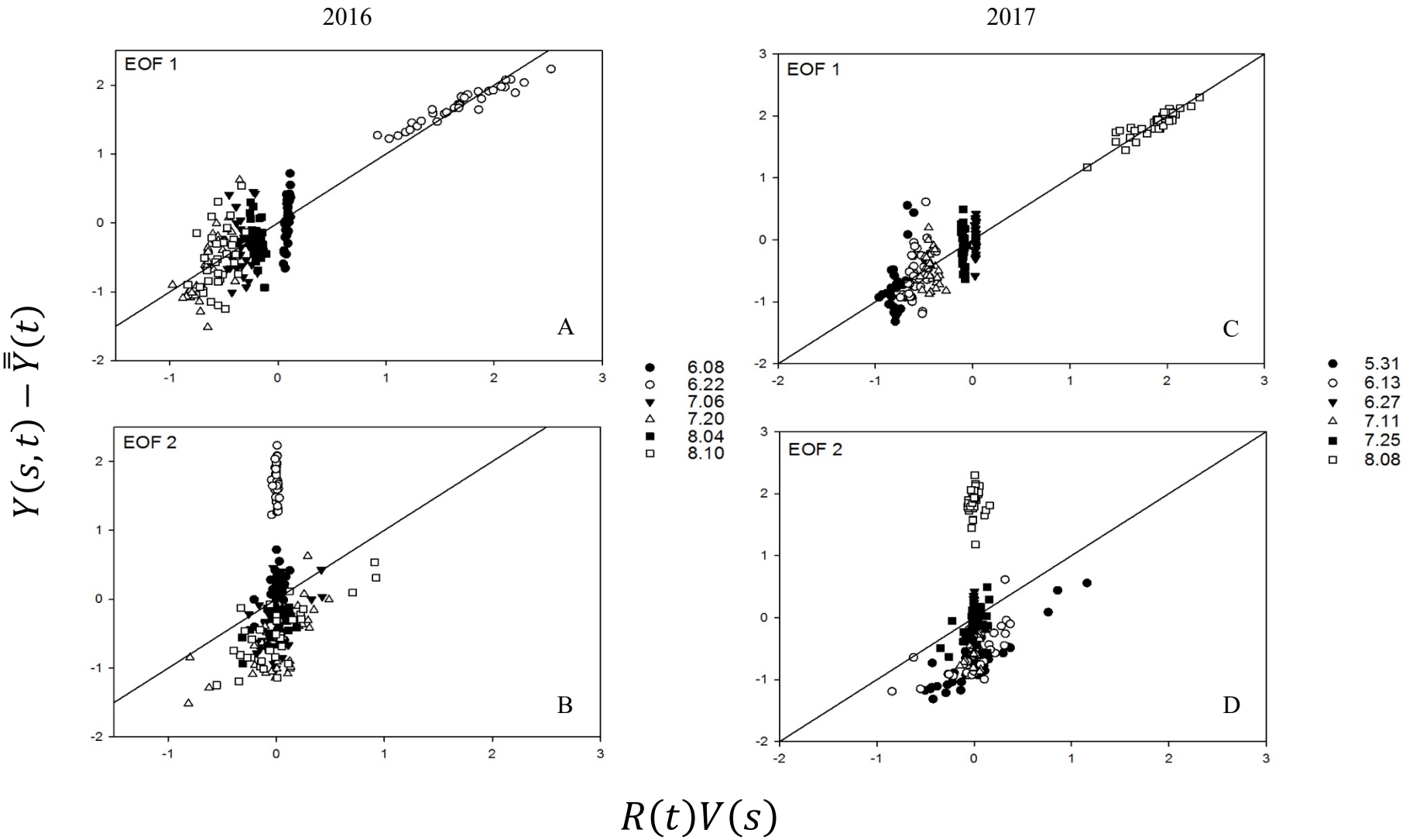


Figure 3.12 First two temporal EOFs of log *E. coli* concentrations for pond P2. (A and B) Temporal EOFs for 2016 separated by sampling date on the right. (C and D) Temporal EOFs for 2017 separated by sampling date on the right.

Precipitation and E. coli concentrations

Precipitation measurements on sampling days and antecedent days were gathered from Weather Underground historical data (Tables 3.6 and 3.7). Precipitation events, resulting in rainfall amounts higher than monthly average, prior to sampling days in pond P2 corresponded with a 10^3 increase in *E. coli* concentrations for both sampling years (June 22, 2016 and August 8, 2017).

Table 3.6 Geometric mean and statistical threshold value of *E. coli* concentrations and precipitation measurements (antecedent and day of sampling) of pond P1

	30-May-16	**31-May-16**	**12-Jun-16**	**13-Jun-16**	**26-Jun-16**	**27-Jun-16**	**10-Jul-16**	**11-Jul-16**	**24-Jul-16**	**25-Jul-16**
Precipitation (mm)	0	0	0	0	0	0	0	0	0	0
GM (CFU 100ml-1)	N/A	1.3	N/A	3.92	N/A	22.9	N/A	9.99	N/A	12.5
STV (CFU 100ml-1)	N/A	4.01	N/A	8.45	N/A	55.3	N/A	24	N/A	27.3

	6-Jun-17	**7-Jun-17**	**20-Jun-17**	**21-Jun-17**	**4-Jul-17**	**5-Jul-17**	**17-Jul-17**	**18-Jul-17**	**31-Jul-17**	**1-Aug-17**	**15-Aug-17**	**16-Aug-17**
Precipitation (mm)	0	0	0	0	8.89	5.84	82.55	0	0	0	16.26	0.254
GM (CFU 100ml-1)	N/A	41	N/A	59	N/A	33.4	N/A	33.5	N/A	67.7	N/A	59.8
STV (CFU 100ml-1) and	N/A	81.3	N/A	141.3	N/A	125.9	N/A	102.3	N/A	94.8	N/A	123

Table 3.7 Geometric mean and statistical threshold value of *E. coli* concentrations and precipitation measurements (antecedent and day of sampling) of pond P2

	7-Jun-16	**8-Jun-16**	**21-Jun-16**	**22-Jun-16**	**5-Jul-16**	**6-Jul-16**	**19-Jul-16**	**20-Jul-16**	**3-Aug-16**	**4-Aug-16**	**9-Aug-16**	**10-Aug-16**
Precipitation (mm)	0	0	18.29	0	10.4	0	0	0	0	0	0	0
GM (CFU 100ml-1)	N/A	26.9	N/A	1183	N/A	10.3	N/A	4.99	N/A	14.1	N/A	6.26
STV (CFU 100ml-1)	N/A	57	N/A	1660	N/A	45.9	N/A	44.22	N/A	26.2	N/A	47.1

	30-May-17	**31-May-17**	**12-Jun-17**	**13-Jun-17**	**26-Jun-17**	**27-Jun-17**	**10-Jul-17**	**11-Jul-17**	**24-Jul-17**	**25-Jul-17**	**7-Aug-17**	**8-Aug-17**
Precipitation (mm)	0	0	0	0	0	1.78	0	0	0.76	0	26.67	1.27
GM (CFU 100ml-1)	N/A	2.55	N/A	3.75	N/A	16.7	N/A	5.57	N/A	12.1	N/A	1150
STV (CFU 100ml-1)	N/A	14.5	N/A	20.9	N/A	47.9	N/A	12.9	N/A	31.06	N/A	1533

Discussion

Temporal stability in the spatial pattern of *E. coli* concentrations was evident in both ponds studied over each sampling season. Locations in the interior of the ponds consistently had significantly lower concentrations than sampling locations on the banks (Figure 3.1). This agreed with previous work from Whitman and Nevers (2003) which found that *E. coli* concentrations were highest in foreshore sand and decreased with increasing water depth. Average depths at both ponds were 2.7 meters in the interior and less than 0.5 meters near the banks. Correspondingly, the MRD ranks for turbidity were higher on average along the banks than the interior for both ponds. Suspended matter and turbidity decrease light penetration which in turn can decrease the solar inactivation by UV radiation of *E. coli* (Pommepuy et al., 1992). Increased turbidity along the banks may have provided shading promoting *E. coli* survival. In addition, we noted that pond banks were shaded by trees and large shrubs. In pond P1, turbidity had a positive correlation with *E. coli* concentrations in the 2017 sampling season (Table 3.3). Droppo et al. (2010) found that indicator organisms were significantly correlated with suspended solid concentrations. Bacteria may often use suspended solids as a place for attachment, a source for food in the form of dissolved organic carbon, and a buffer against environmental stresses or predation (Droppo et al., 2010).

Higher *E. coli* MRD ranks near locations 1,8,7 and 22 in Pond P1 in 2017 (Figure 3.1A) could be attributed to recreational activity which was evident in 2016 sampling season as well. These areas were commonly used for human and animal wading. Wright et al. (2009) found that dogs had the highest contribution of animal

source fecal coliform Enterococci in a recreational beach area. In addition, *E. coli* concentrations have been reported to have prolonged survival in sediments compared to water; and human recreational activity can resuspend sediments. In 2016, however, it was found that sediment samples taken from these two ponds contained low *E. coli* cell counts (Pachepsky et al., 2017). In pond P1 the outflow location (sampling location 6) had higher than the average concentrations of *E. coli* in 2016 and 2017 sampling seasons perhaps due to shading from surrounding trees. The pond P2 also had higher than average *E. coli* concentrations at inflow and outflow locations in 2016 however this observation was not seen in 2017 sampling season. Jenkins et al. (2012) noted that concentrations of fecal indicator bacteria were significantly less at outflow locations than the inflow locations possibly due to longer residence time increasing bacterial exposure to UV-radiation.

Environmental covariates demonstrated patterns of spatial stability (Figure 3.4). Temperatures were generally higher in the interior surface water locations of the ponds for possibly due to the lack of shading that was evident along the pond banks. There were negative correlations between temperature MRD ranks and the MRDs ranks of *E. coli* in both ponds. Blaunstein et al. (2013) showed that inactivation rates of *E. coli* increased with an increase in temperature of agricultural waters. The temperatures in the agricultural water ranged from 10-30°C (Blaunstein et al., 2013). Surface water temperatures taken at sampling times (8:00am-12:00pm) in both ponds and sampling years ranged from 20-30°C. In pond P2 dissolved oxygen MRD ranks were higher within the interior than the banks. This difference could be attributed to the higher concentration of *E. coli* along the pond banks consuming dissolved

oxygen. MRD ranks for pH were negatively correlated with *E. coli* MRD ranks in pond P2. In a microcosm experiment, Parhad and Rao (1974) found that *E. coli* concentrations decreased with higher pH levels in water. The higher pH levels could be explained by the function of algae in an aquatic ecosystem. During photosynthesis algae convert dissolved CO_2 into oxygen thus decreasing the dissolved CO_2 available to form carbonic acid (Zang et al., 2010). Decreasing carbonic acid will increase the pH of the water making the water more basic. On May 31, 2017 the pH was the highest of the sampling days at 9.3 and chlorophyll-a concentrations were the second highest of the season at 63.3 ug/liter (Table 3.2). Higher concentrations of algae could have increased the pH thus decreasing the concentrations of *E. coli*. Photosynthesis occurs to a greater extent when the optimal growth temperature of cyanobacteria, between 20 and 30°C, and chlorophytes, between 20 and 35°C, is reached (Konopka and Brock, 1978; Lurling et al., 2012). The average temperature of the ponds during the time we sampled, 8:00 to 12:00, ranged from approximately 22 to 31°C (Tables 3.1 and 3.2). With higher temperatures promoting photosynthesis, the positive correlation between pH and temperature MRD ranks in pond P2 can be explained as well.

An analysis of the spatial EOFs indicated a large spread of variation over several EOFs for both pond P1 and P2. Empirical Orthogonal Functions seek to explain the maximum amount of variance in the first few EOFs (Hartman, 2016). For pond P1 the first two EOFs for 2016 and 2017 account for only 65% of the total variance which indicates that variance is not easily explained in the first few EOFs. In Figure 3.5 the EOF 1 and EOF 2 pond P1 bar graphs have similar peaks and are

essentially accounting for the same amount of variance, indicating the data variance is uniformly spread among several EOFs. In Figure 3.5B the heights of the bars in EOF 2 are slightly smaller than the height of the bars in the first EOF, Figure 3.5A, indicating the first EOF is accounting for a majority of the total variance. The pond P2 2017 data follows a similar variance pattern to pond P1. The product of the first two spatial EOFs and their respective amplitude functions for both ponds in relation to the observed data centered about the spatial average at each location do not seem to follow a 1:1 ratio as the equation in the methods would imply (Figures 3.6 and 3.7). The data points are tightly clustered and not even spread around the l:1 line. This indicates that the spatial variance for pond P1 was widespread. Interior locations exhibited a larger amount of variance than exterior locations which may be attributed to wind movement along the pond surface or irrigation pulling. Amorim et al (2014) noted that wind movement may be a driver of *E. coli* concentrations at beaches. Irrigation pulling occurred 3 out of the 6 sampling dates in 2017 for pond P2 where 2 of the sampling days with irrigation pulling had the smallest standard errors of log *E. coli* concentrations (July 11th and July 27th in Table 3.2).

Temporal EOFs for pond P1 indicated smaller patterns of temporal variance for 2016 log *E. coli* data than for 2017 log *E. coli* data. In Figure 3.9A the bar graphs are much higher than the second EOF graphs in Figure 3.9B, indicating that the analysis was able to capture the majority of the variance in the first EOF for pond P2 for 2016. The data points for 2016 EOF 1 (Figure 3.11) are closely fit around the 1:1 ratio line and the patterns of data for each sampling date correspond to the log *E. coli* average concentrations in Table 3.1. The most positive values on the Figure 3.11A

graph correspond to the days with the highest concentrations of log *E. coli* and the most negative values correspond to the days with the lowest log *E. coli* concentrations. The 2017 first EOF bar graph (Figure 3.9C) did not capture the majority of the variance which is evident in Table 3.5 where the first temporal EOF for 2017 only accounts for 39.8% of the total variance. This is also evident in Figure 3.11C where the data points do not fit the 1:1 ratio line. In 2017, three out of the six sampling days occurred after a large rain storm (Table 3.6). In 2016 there were no precipitation events prior to sampling days. Precipitation could have affected temporal variance in 2017.

For pond P2, temporal EOFs patterns were less variable than spatial EOFs. The first EOFs for pond P2 2016 and 2017 accounted for the majority of the total variance, indicating the data exhibit a strong temporal pattern which is captured by the EOF analysis. The heights of the bar graphs in Figure 3.10A and C are much higher than the heights of the bar graphs in C and D indicating that the first EOF accounted for the most temporal variability. The data points in Figure 3.12 A and C follow the concentration trends of log *E. coli* values noted in Table 2.2 and 3.2. The highest average log *E. coli* concentration for 2016 in pond P2 occurred on June 22nd (Table 2.2) and the lowest on July 20th. The data points for June 22nd in Figure 3.12A are the most positive values while the data points for July 20th are the most negative. Log *E. coli* concentrations for 2017 in pond P2 correspond to the values in Figure 3.12C where the highest concentration has the most positive data values. The vertical clustering of the data points in Figure 3.12A and C are not explained by the data but may be contributed to pond irrigation pumping on sampling dates.

Conclusion

Spatial differences in *E. coli* concentrations between sampling locations were observed and these differences seemed to be maintained temporally throughout each sampling season. The interior of the ponds had consistently lower *E. coli* concentrations than the bank sampling locations. Sampling water from areas with higher concentrations of indicator bacteria or lower concentrations of indicator bacteria may provide a skewed understanding of the overall microbial water quality for a pond. Thus, for water quality regulatory criterion, it would be favorable to sample bank locations to provide an overestimate of *E. coli* concentrations than to sample interior locations which have lower concentrations of *E. coli.* It may also be valuable to place irrigation outflow pipes near the interior of the ponds where the concentrations of *E. coli* are lower.

Correlations between environmental covariates and *E. coli* concentrations may provide insight into *E. coli* survival patterns. *E. coli* was found to be positively correlated with turbidity in pond P1. Turbidity is often increased after hydrological events when sediment is resuspended in the water table. *E. coli* concentrations were 10^3 times larger than average in pond P2 after hydrological events. Monitoring *E. coli* concentrations in resuspended sediments after hydrological events may provide insight into *E. coli* concentrations dynamics within a pond. In addition, temperature and dissolved oxygen had negative correlations with *E. coli* concentrations in pond P1. Perhaps allowing for longer retention times of irrigation water will increase temperature inactivation of *E. coli* concentrations

Large spatial variance of *E. coli* concentrations may increase the difficulty of finding a single representative location to sample for water quality regulations. Spatial variations of the log *E. coli* concentrations for 2016 and 2017 were largely spread according to EOF analysis in pond P1. The majority of Pond P2 2016 and 2017 spatial variation of the log *E. coli* concentrations were explained in fewer EOFs than pond P1. More sample variance existed over time in the pond interiors versus the bank sampling locations. Temporal analysis indicated that spatial patterns are consistent throughout the sampling season. Once a spatial understanding of *E. coli* concentration variation for a growing season is established, this pattern should remain consistent throughout that particular growing season.

In order to properly regulate and monitor microbial water quality, spatial and temporal *E. coli* concentration variation must be understood. The results of this work show that without thorough sampling, an accurate representation of an irrigation pond water source microbial quality may not be achieved. This work appears to be important in monitoring design and implementation for regulation.

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Thesis Conclusion Summary

The purpose of this thesis was to test whether spatial and/or temporal patterns of *E. coli* concentrations exist in two Maryland irrigation ponds over two summer growing seasons. For both sampling year, 2016 and 2017, the pond interiors of both ponds had persistently lower *E. coli* concentrations than the areas near the banks. Areas used for recreational activities contained higher than average *E. coli* concentrations. When sampling for microbial water quality in irrigation ponds it may be beneficial to sample along the banks of the pond as those locations may provide an overestimate of *E. coli* concentrations. It may also be valuable to place irrigation outflow pipes near the interior of the ponds where the concentrations of *E. coli* are lower.

The log *E. coli* concentration mean relative differences in 2017 were highly correlated with several environmental covariates (pH, DO, temperature, and turbidity) which may have contributed to spatial patterns of *E. coli* concentrations. Knowledge of *E. coli* concentration correlation with environmental covariates may be used to understand *E. coli* survival patterns. Precipitation events prior to sampling days in Pond P2 had a dramatic impact on *E. coli* concentrations. However, in Pond P1, precipitation events exhibited less of an impact on *E. coli* concentrations perhaps due to the fact that pond P2 was directly stream fed while pond P1 was connected to a retention pond. Sampling after a precipitation event may provide knowledge of understanding *E. coli* concentration seasonal dynamics.

Spatial variations of the log E. *coli* concentrations were more variable, according to Empirical Orthogonal Analysis, than temporal variations. The majority

of the total temporal variance of log *E. coli* concentrations for both ponds were explained in the first two temporal EOFS. It is preferred when the maximum amount of variance is explained in the first few EOFs (Hartman, 2016). Spatial EOF analysis indicated a large spread of variation over several EOFs for both pond P1 and P2. Empirical orthogonal analysis of log *E. coli* concentrations suggests that there is spatial variation among sampling locations, yet those variations are maintained within each sampling year. Further analysis using EOF should be conducted to understand the spatial variance of *E. coli* concentrations.

Another purpose of this study was to test whether chlorophyll-*a* concentrations were correlated with *E. coli* concentrations. In 2016, pond interior sampling location MRD ranks were highly negatively correlated with log *E. coli* MRD ranks in pond P2. Data collected during the 2017 sampling year and ponds did not support the hypothesis that chlorophyll *a* and *E. coli* concentrations were correlated. Although several studies have indicated a favorable interaction between algae/cyanobcteria and *E. coli* (Engelbert et al., 2008; Byappanahalli et al., 2003; Cole, 1982), further monitoring to understand *E. coli* and algae dynamics in irrigation ponds should be conducted.

The results of this work show that without understanding the distribution of *E. coli* concentrations spatially and temporally in irrigation ponds an accurate representation of microbial water quality cannot be achieved. Furthermore, there are several environmental covariates that may affect *E. coli* distribution/survival and should be considered when monitoring *E. coli* concentrations. Properly monitoring

and regulating irrigation water quality seems to be an area where further research needs to be conducted.

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