EFFECTS OF RESIDENTIAL DEVELOPMENT ON RESERVOIR WATER QUALITY AND EFFECTIVENESS OF CHLOROPHYLL-A MONITORING TECHNIQUES

by

WESLEY GERRIN

(Under the Direction of Susan Wilde)

ABSTRACT

Human activities, such as residential development, urbanization, and deforestation can have profound effects on water quality in streams, lakes, and oceans worldwide. Water is a critical resource and must be managed and protected as such. Monitoring and understanding human effects on water resources is necessary for maintaining usable water supplies. Our studies describe both the effects of residential development on water quality in a major drinking water source and an attempt to improve the capacity of practitioners to monitor their respective drinking water sources. Specifically, we assess the potential effects of onsite wastewater treatment on reservoir water quality and evaluate the accuracy of in-situ chlorophyll-a monitoring techniques. Based on our results, the main source of water quality variation in our study area was linked to precipitation events and is most likely stormwater or surface runoff. We also propose a method for improving the accuracy of in-situ chlorophyll-a fluorescence data.

INDEX WORDS: Onsite wastewater treatment, Septic tank system, Residential development, Lake Lanier, Eutrophication, Harmful algal blooms

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Onsite wastewater treatment systems (OWT), more commonly known as septic systems, are used widely for disposal and treatment of domestic wastewater in suburban and rural settings that lack access to centralized wastewater treatment facilities via sanitary sewer. OWT relies on anaerobic digestion of organic matter in a bioreactor (septic tank; Figure 1) with subsequent aerobic treatment by soil percolation (Beal et al. 2005, Gill et al. 2009) (Figure 2).

The United States Environmental Protection Agency (USEPA) lists pathogens—including those associated with human fecal contamination—and excess nutrients as the two leading causes of water quality impairment in the United States (USEPA 2015). It is often difficult to quantify and isolate all sources of impairment within a watershed, so these impairments cannot all be attributed to OWT pollution. However, some information exists to suggest at least a partial connection. OWT systems have been shown to be a significant source of human fecal pollution in streams in highly developed areas of Georgia (Sowah et al. 2017), suggesting that these pollutants can be transferred to other environments via both groundwater and stream connections.

In a water supply reservoir setting, contributing watersheds with high density OWT systems have been shown to export significantly higher levels of nutrients than watersheds with low density or no OWT systems (Iverson et al. 2018). Not only do excess nutrient inputs from OWT systems occur via groundwater, making them non-point-source pollutants, there have been documented cases in which OWT systems were designed so poorly that they became point-source inputs (Jarvie et al. 2010). However, information is relatively scarce elucidating similar effects in reservoirs in the Piedmont region of Georgia.

Factors that may affect the ability of OWT to remove nutrients prior to entry into a waterbody include location, age, frequency of maintenance, and distance from the waterbody (Withers et al. 2012). Unfortunately, documentation of important data related to OWT installation and maintenance is inconsistent, leading to uncertainty and difficulty using only groundwater and septic tank data to extrapolate effects to areas of a reservoir that are not sampled. Lack of accurate documentation makes estimating accurate total loads from septic systems to waterbodies difficult (Withers et al. 2012).

Although USEPA directly lists nutrients as a source of impairment, other problems such as harmful algal blooms (HABs) and excessive aquatic vegetation can be indirect effects associated with excess nutrient loading (USEPA 2015). Coastal and estuarine environments with high density development are receiving excess nutrients from OWT systems (Harman et al. 1996, Humphrey et al. 2015), which have been linked to consequent HABs (Lapointe et al. 2017).

Background Information

Onsite Wastewater Treatment

The objective of soil percolation is to remove excess nutrients, such as phosphorus and nitrogen, from the wastewater before it enters waterbodies via groundwater. The soil percolation process is especially important for homes that are built near reservoirs and streams and have very little distance between the OWT system drain field and the edge of the waterbody. Anaerobic digestion and soil percolation are generally effective at removing nutrients from groundwater, but there are factors that can render these processes much less effective. One of the main requirements for efficient treatment of wastewater using OWT is periodic maintenance, including checking tanks for leaks, making sure there are no clogs in the tank or drain field, and having the tank pumped out to ensure it does not fill and overflow. Failure to complete regular OWT system maintenance may result in backups of wastewater on the surface, which can easily reach waterbodies via surface runoff before it can be properly treated by the soil. Another important consideration regarding OWT effectiveness is the location where the systems are placed, as some sites have characteristics that are better for OWT than others. Soil type is an important factor to consider when placing an OWT system, as some soils have much slower percolation rates and higher adsorption capacities than others. Ideal soils have a wide variety of adsorption materials, which include organic matter, clay minerals, and iron hydroxides, and allow for more effective attenuation of phosphorus without creating preferential flow paths (Rea and Upchurch 1980). Sites with high water tables, such as those close to waterbodies, are generally less desirable because soils are likely to become saturated much more quickly, thus diminishing the capability of the soil for percolation and adsorption (Arnade 1999). An additional consideration for sites that are close to streams or reservoirs is the distance from the drain field to the shoreline of the waterbody, as it is important for there to be enough soil in that area to effectively adsorb all nutrients from the wastewater (Jones and Lee 1979, Chen 1988).

Water Quality and Chemistry

Throughout the literature, multiple parameters have been used to investigate OWT related water quality degradation in waterbodies, including nutrients (nitrogen and phosphorus), algal bloom indicators (chlorophyll-a), and fecal pollution indicators (fecal coliform, *E. coli,* human waste DNA markers, etc.). In general, changes in these parameters can be considered a direct or indirect response by the waterbody to the addition of pollutants and can be traced to several different sources, including surface runoff, groundwater, and inflows from streams or other parts of the waterbody. For example, a direct response could be the simple increase in nutrient concentrations resulting from an effluent, while an indirect response could be an algal bloom (increase in chlorophyll-a, pH, and dissolved oxygen) triggered by increased nutrient concentrations. Another useful tool for tracing wastewater effluent is chloride, a water chemistry parameter that is not necessarily indicative of impairment, but that is often found in high concentrations in wastewater effluent (Alhajjar et al. 1990). Multiple studies have shown that chloride can be used to track a wastewater "plume" through both surface water and groundwater (Alhajjar et al. 1990, Harman et al. 1996, Kochary et al. 2017). Unfortunately, these parameters (nitrogen, phosphorus, chlorophyll-a, E. coli, etc.) require resource intensive analysis in a laboratory, which can limit monitoring both spatially and temporally. A limited sampling regime, if not properly allocated, will most likely fail to identify areas of water that are more degraded than others and to pinpoint the source of the pollutants causing the degradation.

Sensor Technology

Various technologies have emerged to attempt to mitigate sampling limitations caused by resource availability. Yellow Springs Instruments (YSI) is a company involved in developing cutting edge technology for monitoring water quality—such as sensors that measure chlorophyll-a and phycocyanin—that combine with platforms measuring more typical water quality parameters (temperature, dissolved oxygen, specific conductance, and pH). In-situ measurement platforms allow for measurement of these parameters in-situ, without involving any of the steps of analysis in a laboratory. However, in-situ sensors require calibration prior to use and certain sensors have shelf-lives before they require total replacement (1 year for pH). Additionally, for various reasons, many of the parameters measured by these sensors must be "post-calibrated" by relating the measurements to values acquired from laboratory analysis of the same water. Once these relationships are established for a given location and date, we may have more confidence in subsequent measurements.

In-Situ Chorophyll-a Measurement

The YSI total algae sensor quantifies the amount of chlorophyll-a or phycocyanin in-situ by measuring the fluorescence of the algal cells in the water. In-situ methods differ from standard chlorophyll-a determination, which disrupts the algal cells, extracts the chlorophyll-a from the cells, and quantifies the concentration of chlorophyll-a in the extract using a fluorometer or spectrophotometer. Although less time intensive, the in-situ method is less accurate than the extraction method and should only be used to supplement laboratory analysis (YSI Environmental 2000).

Another consideration for measuring in-situ fluorescence is a phenomenon called non-photochemical quenching. Non-photochemical quenching is a process by which algal cells depress or "quench" fluorescence as a response to intense light (Muller et al. 2001). According to Müller et. al, quenching effects are demonstrated within seconds after saturation with intense light, but recovery to full fluorescence usually takes minutes. Non-photochemical quenching could affect the relationship between in-situ chlorophyll-a fluorescence measurements and those obtained from a laboratory extraction, and therefore diminish confidence in the in-situ measurements. All considerations regarding in-situ measurements acquired by a sensor under field conditions must be evaluated before these measurements can be considered accurate and acceptable for use in water quality monitoring.

Lake Lanier Description

Lake Sidney Lanier is an 18,000-ha impoundment of the Chattahoochee river located northeast of Atlanta, GA, United States and is operated by the United States Army Corps of Engineers (Figure 3). The reservoir has many uses, including water supply, hydroelectricity, flood control, and recreation. Since its construction in 1956, residential development has occurred along much of the shoreline, resulting in the installation of approximately 14,000 OWT systems within parcels of land directly adjacent to the shoreline (GCDWR *Unpublished Data*).

A reservoir-wide water quality study was done in 2005 to characterize the factors contributing to overall water quality in Lake Lanier. This study found three major contributors to water quality variation in the lake and its tributaries: stormwater runoff, point-source wastewater discharges, and groundwater inputs (Zeng and Rasmussen 2005). These results could suggest that a portion of the groundwater component originates from OWT discharges, but the study did not infer the source of the groundwater inputs.

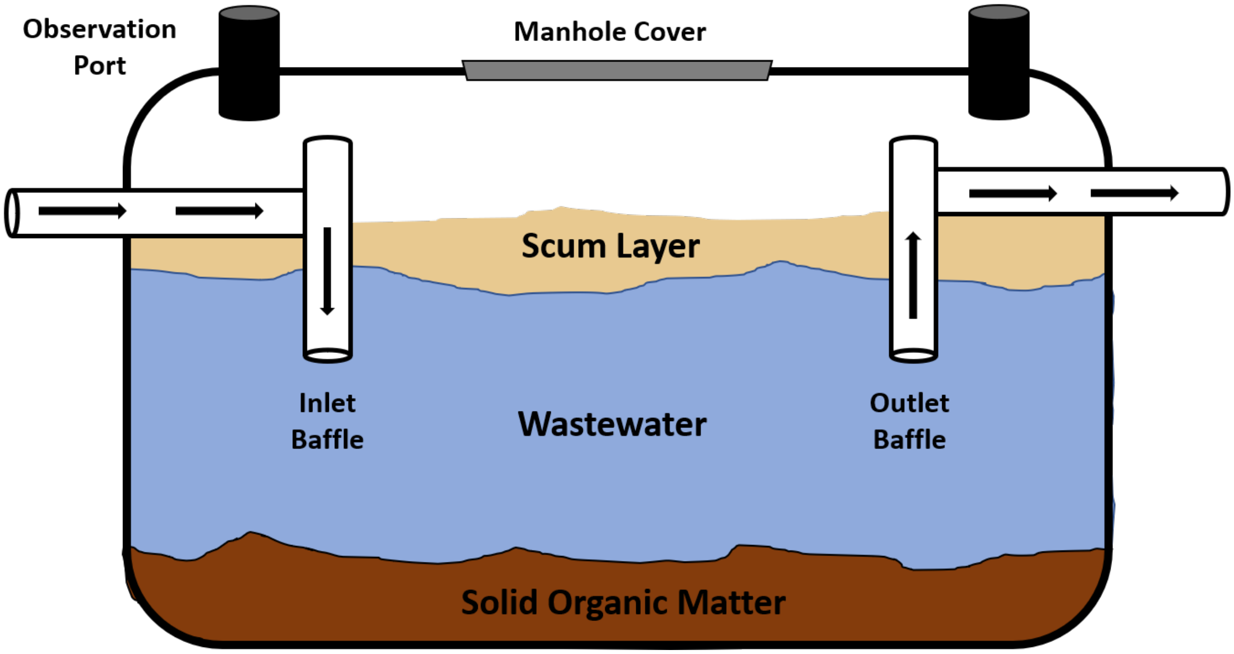

Figure 1.1. Diagram showing the septic tank portion of an onsite wastewater treatment system.

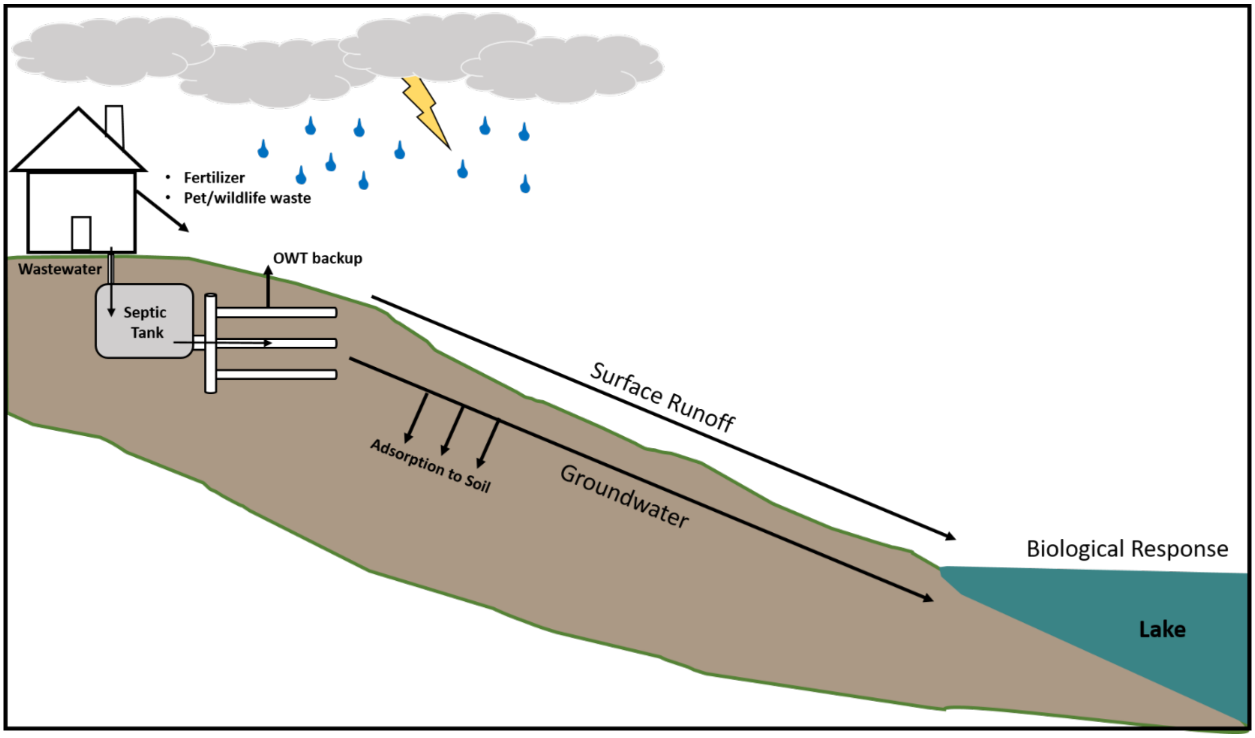

Figure 1.2. Conceptual model showing an entire onsite wastewater treatment system, how the effluent from the tank could potentially interact with the soil and nearby waterbodies, and other potential sources of nutrients.

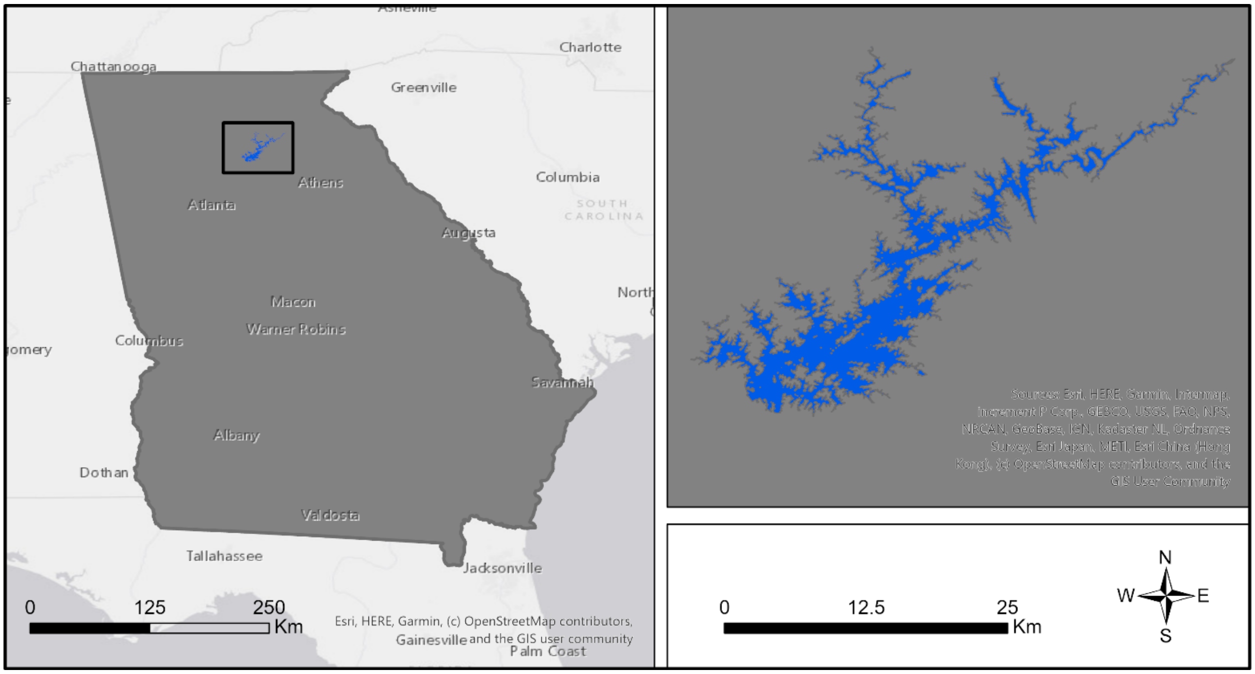

Figure 1.3. Lake Lanier and its location northeast of Atlanta, Georgia, United States.

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CHAPTER 2

EFFECTS OF ONSITE WASTEWATER TREATMENT SYSTEMS ON WATER QUALITY IN A SOUTHEASTERN UNITED STATES PIEDMONT RESERVOIR [1]

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[1] Gerrin, W.L., Montes, C.R., Shelton, J.L., and S.B. Wilde.
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Abstract

Onsite wastewater treatment (OWT) systems are commonly used for treating domestic wastewater in areas that do not have access to sanitary sewer and centralized wastewater treatment facilities. OWT systems include a septic tank that disposes of solid waste and a drain field that treats wastewater via soil percolation. Proper installation of an OWT system allows for sufficient distance and soil dispersal area between the drain field and the nearest waterbody for complete nutrient adsorption. Previous research has shown that high densities of OWT systems can affect water quality in rivers, reservoirs, streams, and estuaries. This study investigates effects of OWT system presence, site characteristics, and precipitation on water chemistry in four coves of Lake Lanier, Georgia, United States. Total phosphorus, total nitrogen, E. coli, chloride, and chlorophyll-a data were collected from May-October 2020 and modeled in a generalized additive model framework using site dispersal area and slope length, along with precipitation, as predictors. A significant difference was found between E. coli concentrations in developed (OWT present) and undeveloped (no OWT) coves. Precipitation was a significant predictor of all water chemistry parameters. We cannot attribute the difference in E. coli concentrations to OWT without DNA source tracking to distinguish between animal and human bacteria. However, we conclude that residential development does affect E. coli concentrations. The significant relationship between precipitation and all tested water chemistry parameters likely indicates that variation in water chemistry is attributable to surface runoff and stormwater rather than OWT systems.

Introduction

Onsite wastewater treatment (OWT) systems are a commonly used method for disposal and treatment of domestic wastewater in rural and suburban areas with limited or no access to sanitary sewer and centralized wastewater treatment facilities. An OWT system consists of a septic tank, in which all solid waste is anaerobically digested, and a drain field, where nutrient-rich wastewater is treated aerobically through soil percolation (Beal et al. 2005, Gill et al. 2009). If properly installed and maintained, OWT systems should remove excess phosphorus and nitrogen before they enter waterbodies via groundwater. Proper installation of an OWT system includes allowing sufficient distance between the drain field and the edge of a waterbody for complete phosphorus adsorption (Jones and Lee 1979, Chen 1988), selection of sites with enough area of soils that have a variety of adsorption materials (organic matter, clay minerals, and iron hydroxides) and that allow for attenuation of phosphorus without preferential flow paths (Rea and Upchurch 1980), and selecting sites that do not have high water tables that could become saturated more quickly and diminish percolation and adsorption capability (Arnade 1999). Proper maintenance of an OWT system includes periodically checking the tank for leaks, making sure there are no clogs in the tank or drain field, and having the tank pumped out to ensure that it does not fill and overflow. If a tank or drain field backup occurs, wastewater may reach the surface and enter waterbodies via overland flow before being properly treated by the soil (Withers et al. 2012).

Previous studies have shown that OWT systems are a source of human fecal pollution in streams (Sowah et al. 2017) and that watersheds with high densities of OWT systems export significantly higher levels of nutrients than watersheds with low density or no OWT systems (Iverson et al. 2018). Areas with high densities of OWT systems have also been linked to excess nutrient pollution and harmful algal blooms in coastal and estuarine environments (Humphrey et al. 2015, Lapointe et al. 2017). Therefore, existing literature suggests that there could be a connection between lake water quality and OWT systems being employed by homes along lake shorelines. Previous studies that have investigated the effects of OWT systems on lake water quality have focused on tracking pollutants through groundwater wells, not on the potential responses occurring in the lake itself (Jones and Lee 1979, Chen 1988, Kochary et al. 2017).

The overall goal of this study was to determine whether OWT systems effect lake water quality and whether that effect could be discovered within the water along a reservoir shoreline. Specific objectives of this study were to (1) collect water chemistry samples from sites along the shoreline of developed and undeveloped coves, (2) investigate the differences in water quality/chemistry parameters between developed and undeveloped coves, and (3) use terrain analysis and other environmental data to investigate relationships with constituents that can be present in wastewater, specifically chloride, phosphorus, nitrogen, and E. coli, as well as chlorophyll-a, which represents the biological response in the lake. Our expectation is that if OWT systems affect lake shoreline water quality, we will discover higher concentrations of wastewater constituents in developed coves than in undeveloped coves. We also expect that if higher concentrations of wastewater constituents exist in relation to sites with shorter slope length and smaller dispersal area between the shoreline and the associated OWT system, water chemistry may be affected by poorly sited OWT systems more than others. Finally, if water chemistry shows a strong relationship with precipitation, we would likely conclude that water chemistry changes are due to inputs from surface runoff or stormwater rather than OWT systems.

Materials and Methods

Study Location

Lake Sidney Lanier (Lake Lanier) is an 18,000-ha impoundment of the Chattahoochee river located northeast of Atlanta, GA, United States and is operated by the United States Army Corps of Engineers (figure 1). Designated uses of this reservoir include water supply, hydroelectricity, flood control, and recreation. Lake Lanier was constructed in 1956 and has since undergone extensive residential development along its shoreline.

The coves selected for this study included two coves with extensive residential development (coves DC1 and DC2; figure 1), in which most if not all of the homes employ OWT systems for wastewater treatment, and two coves that were undeveloped at the beginning of the study (coves UC1 and UC2; figure 1). A camping area was constructed on the shoreline of cove UC1 during our study, and we are unsure of its wastewater handling methods.

Data Collection

Water Chemistry Parameters

Water sampling occurred monthly from May-October 2020 at 10 sites per cove in all four coves (figure 2). Water chemistry samples were collected using a typhoon submersible pump from 1-m depth and approximately 1-2 meters from the shoreline if boat access was possible; otherwise, sampling was done as close to the shoreline as possible. Water samples were kept on ice throughout each sampling event and were immediately transported to the Gwinnett County department of water resources laboratory for chemical analyses following the conclusion of sampling (table 1).

Terrain Analysis and Environmental Data

Terrain analysis for the developed coves was done in ArcGIS Pro version 2.7.1. A 10-m resolution digital elevation model (DEM) was developed using Gwinnett county light detection and ranging data. The DEM was inverted, and the resultant DEM was used to calculate the dispersal area from the septic tank most directly uphill from each water sampling site. Exact position of most septic tanks was unknown, so the position of the back of each home was used as a proxy for septic tank location. Dispersal area was calculated from the D8 flow direction raster using the watershed tool in the ArcGIS hydrology toolbox. Slope length was also calculated using the Pythagorean theorem for each of the sites using the position and elevation of the back of each house and sampling site. Fifteen-minute precipitation data were sourced from a nearby United States Geological Survey gage on Buford Dam (US Geological Survey 2020).

Data Analysis

All terrain and environmental data analyses were done within a generalized additive modeling (GAM) framework, a class of generalized linear model which estimates the sum of non-parametric smooth functions instead of assuming a linear relationship with predictors (Hastie and Tibshirani 1999). The GAM framework allows for the identification of linear and nonlinear covariate effects and takes the form of:

$$g(Y_i) = \beta + \sum_{J=1}^{P} f_i\,(X_i)$$

where $g(Y_i)$ is the expectation of the dependent variable, β represents parametric coefficients, and f_i (X_i) represents the variables explained by the non-parametric smooth functions. GAMs were fit using the "mgcv" package version 1.8-33 in R (Wood 2017). GAMs were fit using total phosphorus, total nitrogen, E. coli, chloride, and chlorophyll-a as the response variable in five different models, cove treatment (developed or undeveloped) as a parametric categorical predictor, and slope length divided by dispersal area and precipitation as non-parametric smooth terms. Missingness of terrain data for undeveloped coves, which is simply due to lack of septic tanks, was handled by multiplying the slope length divided by dispersal area smooth term by zero for all instances of missing data within the model. All response variables and terrain variables were log-transformed and precipitation was square-root transformed to meet the assumptions of the Gaussian distribution. Site ID was added to each model as a random effect to control for variability between sites. The "mgcv" package allows for the addition of random effects in a way that represents them as penalized regression terms similar to the other model parameters (Wood 2013). The thin plate spline smoother function was used for all fixed effect smooth terms.

Each model was evaluated using 5-fold cross validation. Using the *createFolds* function in the "caret" package version 6.0-86 in R, the dataset was randomly divided into five equal subsets (i.e., folds). During this procedure, one of the folds was used as a validation dataset, while the other folds were used to train the model. This process was repeated five times so that each fold was used both as a training dataset and a validation dataset. Goodness of fit for each validation fold was determined by calculating the root mean squared error (RMSE). Model and cross-validation RMSE values were compared to evaluate model performance.

Results

According to the parametric cove treatment variable, mean concentrations in undeveloped and developed coves significantly differed ($\alpha = 0.05$) in chloride ($p = 0.003$), E. coli ($p < 0.001$), and total nitrogen ($p = 0.019$) models (figure 3). Total nitrogen and chloride concentrations were significantly higher in undeveloped coves than developed coves and E. coli concentrations were significantly higher in developed coves than undeveloped coves (figure 3). Chlorophyll-a and total phosphorus concentrations did not differ between cove treatments. Adjusted coefficient of determination values (R^2) and percent total deviance explained are presented for each of the five models in table 2. Precipitation significantly affected concentrations of all five parameters ($p < 0.001$), while slope length divided by dispersal area did not significantly affect concentrations of any parameters. The precipitation smooth term had a significant positive affect on total phosphorus (figure 4), total nitrogen (figure 5), and E. coli (figure 6) concentrations, and had a significant negative affect on chloride (figure 7) and chlorophyll-a (figure 8) concentrations. Mean five-fold cross-validation RMSE values were comparable to model RMSE values for all five models, indicating good model fit (table 2).

Discussion

Each of the five water chemistry parameters that were evaluated as a part of this study were only significantly affected by the amount of precipitation that occurred 24 hours prior to sampling. Neither dispersal area nor slope length were related to the concentrations of any of the parameters that we measured. The significant relationship between water chemistry parameters and rainfall, in addition to the lack of relationship between water chemistry and both dispersal area and slope length, suggests that water chemistry changes along the lake shoreline were mainly explained by surface runoff and/or stormwater rather than poorly sited OWT systems.

Perhaps the most compelling result of this study is the significant and substantial difference in E. coli concentrations between developed and undeveloped coves. The methods employed in this study do not allow us to conclude that higher E. coli concentrations are a result of septic system inputs. A DNA tracer study would be required to distinguish human sources of E. coli from animal sources of E. coli before a claim could be made that septic systems are the cause of significantly higher E. coli concentrations in the developed coves. However, whether E. coli sources are animal or human, we can conclude that residential development has both a significant and substantial effect on E. coli concentrations along the lake shoreline.

Although total nitrogen and chloride concentrations are significantly higher in the undeveloped coves than the developed coves, these differences are likely only present because of large sample size and low variability between measurements. Additionally, both the mean and range of total nitrogen concentrations are low within the range of reference conditions (0.30-0.96 mg/L) for the Piedmont ecoregion of the southeastern United States according to United States Environmental Protection Agency ambient water quality criteria recommendations (USEPA 2000), meaning that the difference in total nitrogen concentrations is likely not biologically relevant.

Based on our results, we conclude that the presence of residential development, and therefore onsite wastewater treatment systems, does not affect total phosphorus or chlorophyll-a concentrations along the shoreline of Lake Lanier. We also conclude that the statistical difference between total nitrogen and chloride concentrations in developed and undeveloped coves is most likely due to large sample size and low variation and does not represent a biologically relevant treatment effect. Given the both statistically significant and substantial difference between E. coli concentrations in developed and undeveloped coves, we can conclude that residential development does have an effect on E. coli concentrations in Lake Lanier. However, a DNA tracer study would be needed to further characterize the source of E. coli and potentially attribute the source to humans, and therefore onsite wastewater treatment.

Total phosphorus, total nitrogen, E. coli, chloride, and chlorophyll-a all showed significant relationships with precipitation, and not with dispersal area or slope length of particular sites. This result further suggests that concentrations of the constituents we tested are not affected by either the presence or placement of onsite wastewater treatment systems at a site, but that they are either entering the system via or being affected by surface runoff or stormwater. Our data suggest that the OWT systems and associated soils that were assessed as a part of this study are serving the function of nutrient processing well enough such that there is no measurable effect on shoreline water quality. However, given that we do not have reliable information about the management regime or condition of the OWT systems at our sites, we cannot conclude what effect a failing or leaky septic tank or drain field may have on shoreline water quality. We also cannot conclude whether the surface runoff signal present in our models includes input from leaky septic tanks or drain fields backing up to the surface.

Future studies attempting to elucidate effects of OWT on shoreline water quality should focus on identifying sources of E. coli as animal or human in addition to the collection of nutrient and biological response data. This additional data would allow for a much more definitive conclusion as to whether or not nutrient inputs are related to wastewater or other sources.

Table 2.1. Parameters that were measured monthly at a total of 40 sites within four coves in Lake Lanier, Georgia from May-October 2020 and the instruments and methods employed to analyze samples under laboratory conditions.

Parameter	Instrument	Method
Chloride	Ion Chromatograph	EPA: 300.1
Chlorophyll-a	Spectrofluorometer	EPA: 445.0
Total Phosphorus	Segmented Flow Analyzer	EPA: 365.2
Total Nitrogen	Segmented Flow Analyzer	SM: 4500
E. coli	Idexx	SM: 9223

Table 2.2. Model evaluation parameters for each of the five models used to establish relationships between water chemistry and both terrain and environmental data.

Model	Adjusted R^2	Deviance Explained (%)	Model RMSE	Mean 5-fold CV RMSE
Total phosphorus	0.304	35.50	0.276	0.272
Total nitrogen	0.741	74.60	0.119	0.118
E. coli	0.339	35.40	1.265	1.259
Chloride	0.090	10.10	0.045	0.041
Chlorophyll-a	0.565	57.30	0.282	0.282

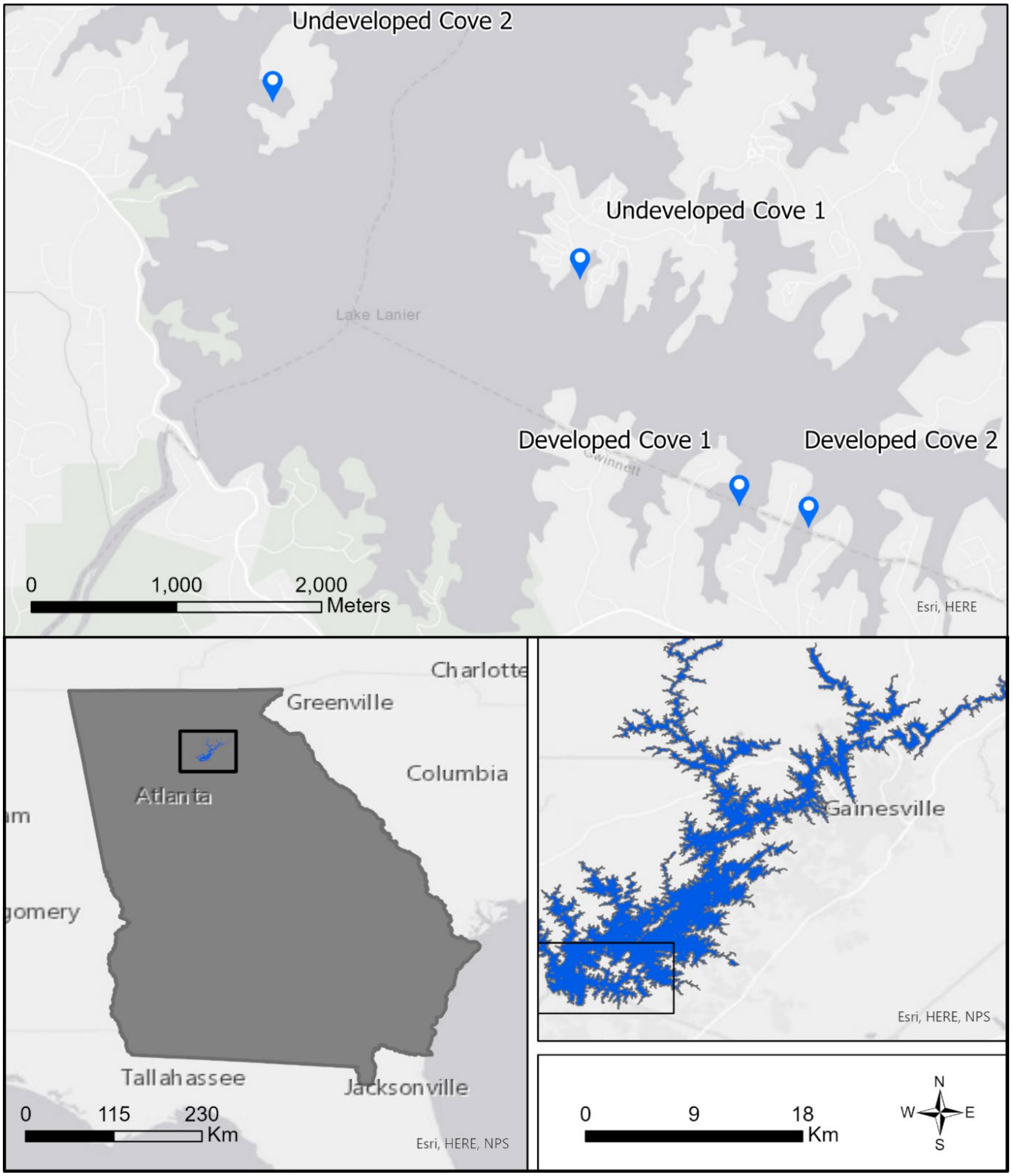

Figure 2.1. Locations of the four coves that were used to evaluate the effects of onsite wastewater treatment systems on water quality in Lake Lanier, Georgia and their relative locations within the entire lake.

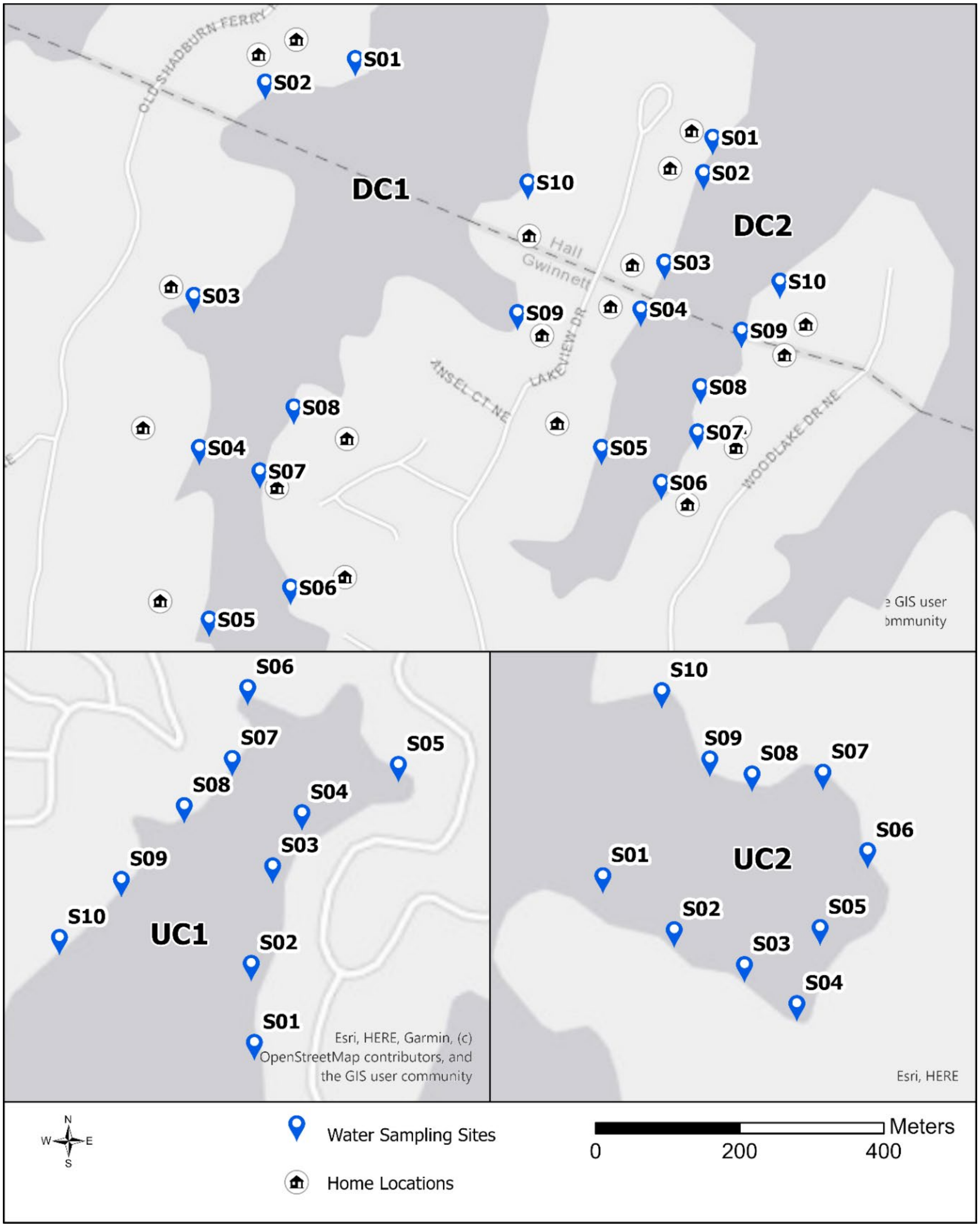

Figure 2.2. Locations of each sampling site and home location (DC1 and DC2 only) within each of the four coves that were used to evaluate the effects of onsite wastewater treatment systems on water quality in Lake Lanier, Georgia.

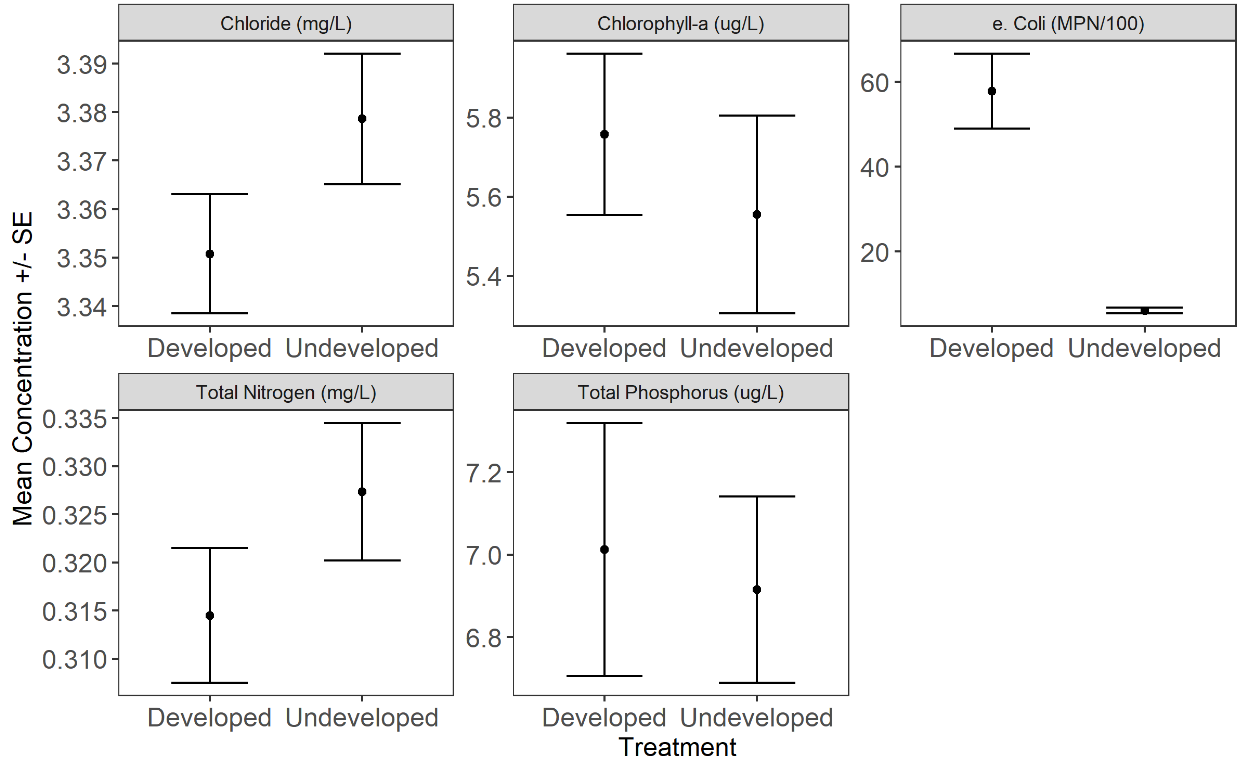

Figure 2.3. Mean concentrations (± standard error) of chloride (mg/L), chlorophyll-a (µg/L), E. coli (MPN/100), total nitrogen (mg/L), and total phosphorus (µg/L) by cove treatment (developed or undeveloped) in samples taken from Lake Lanier, Georgia from May-October 2020.

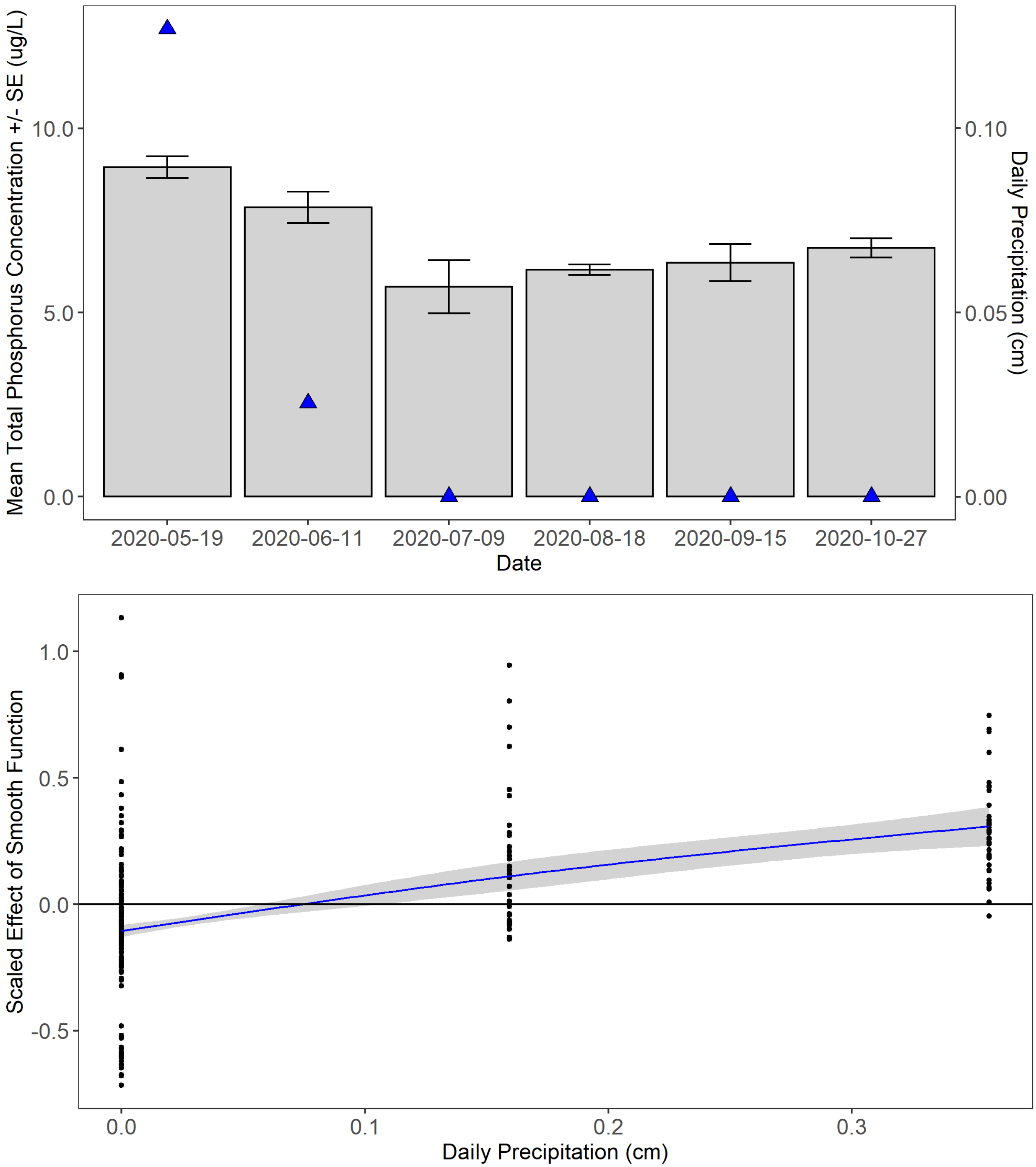

Figure 2.4. Mean concentrations (± standard error) of total phosphorus (µg/L) and total rainfall amounts by sampling date (top), and scaled effect of the daily precipitation model smooth function on total phosphorus concentration (bottom) in samples taken from Lake Lanier, Georgia from May-October 2020.

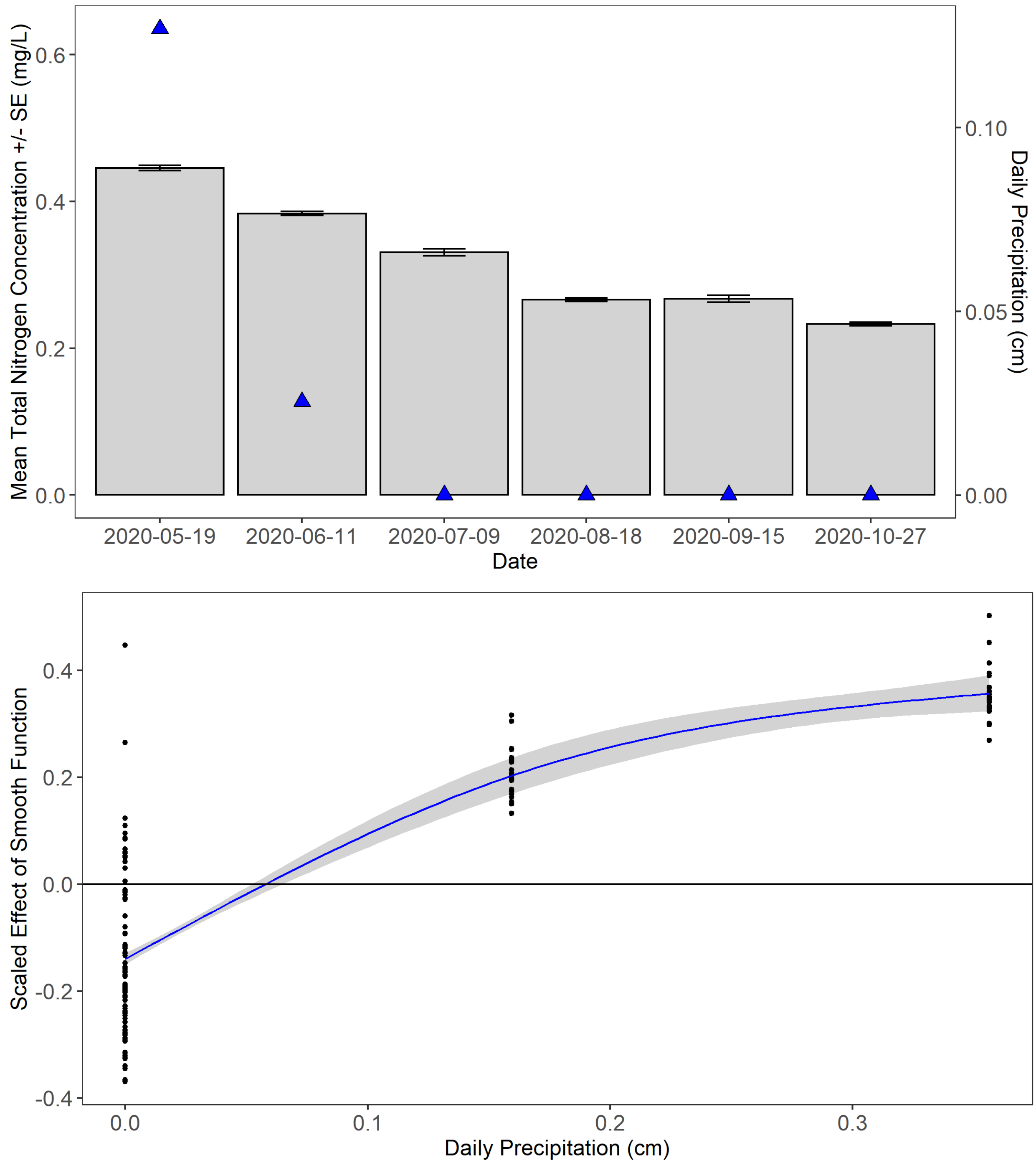

Figure 2.5. Mean concentrations (± standard error) of total nitrogen (mg/L) and total rainfall amounts by sampling date (top), and scaled effect of the daily precipitation model smooth function on total nitrogen concentration (bottom) in samples taken from Lake Lanier, Georgia from May-October 2020.

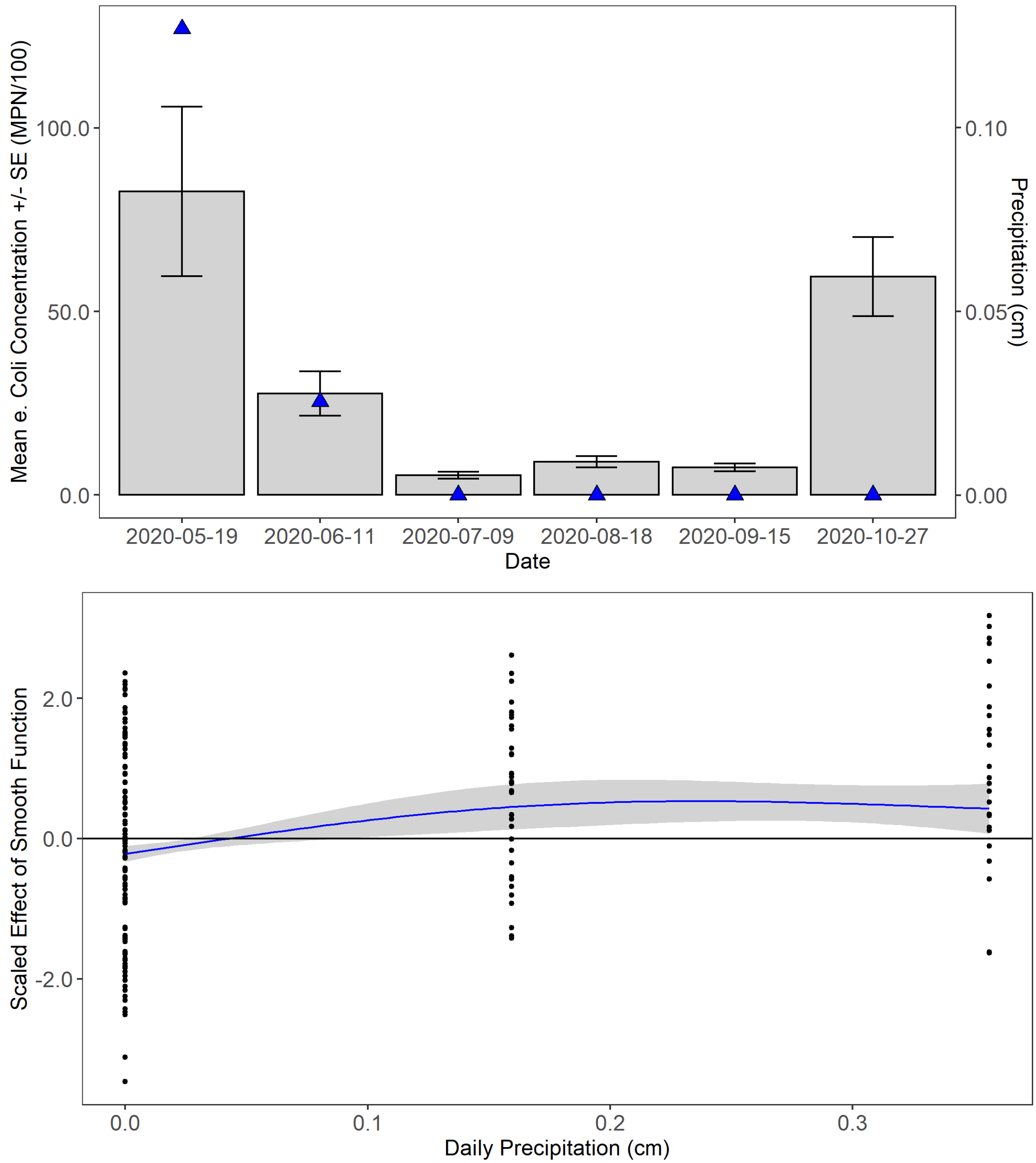

Figure 2.6. Mean concentrations (± standard error) of E. coli (MPN/100) and total rainfall amounts by sampling date (top), and scaled effect of the daily precipitation model smooth function on E. coli concentration (bottom) in samples taken from Lake Lanier, Georgia from May-October 2020.

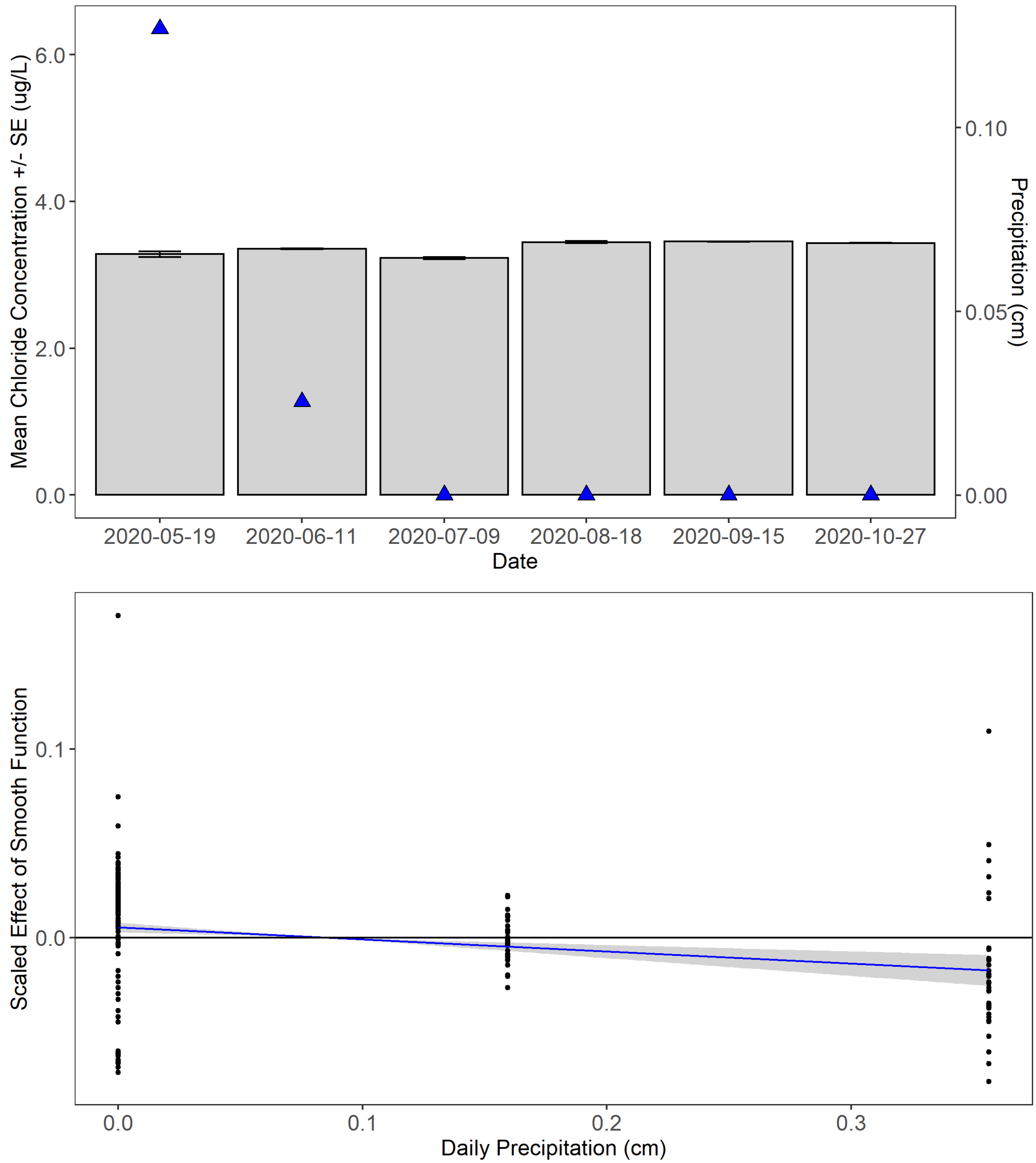

Figure 2.7. Mean concentrations (± standard error) of chloride (mg/L) and total rainfall amounts by sampling date (top), and scaled effect of the daily precipitation model smooth function on chloride concentration (bottom) in samples taken from Lake Lanier, Georgia from May-October 2020.

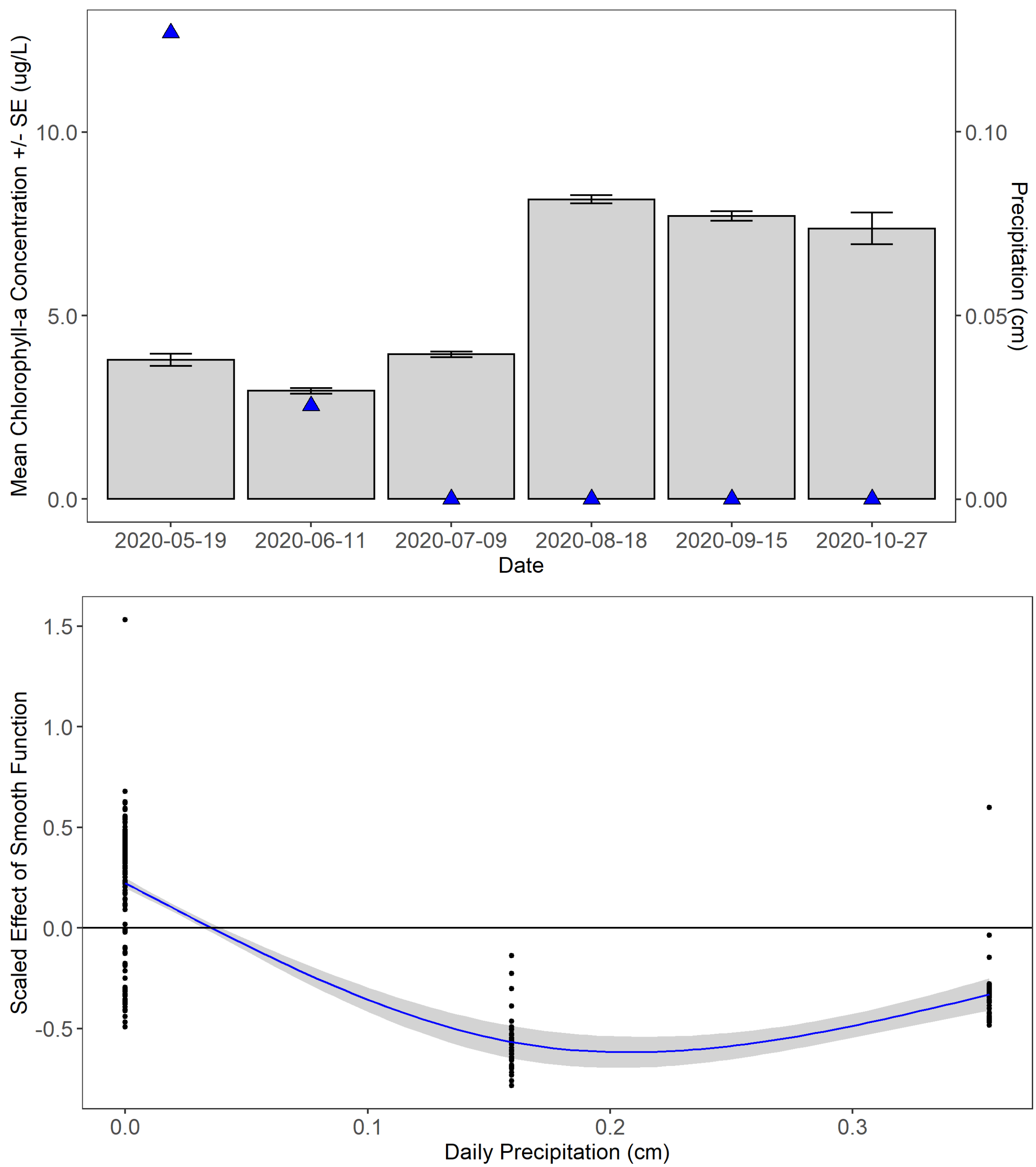

Figure 2.8. Mean concentrations (± standard error) of chlorophyll-a (µg/L) and total rainfall amounts by sampling date (top), and scaled effect of the daily precipitation model smooth function on chlorophyll-a concentration (bottom) in samples taken from Lake Lanier, Georgia from May-October 2020.

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CHAPTER 3

EFFECTS OF ENVIRONMENTAL CONDITIONS ON IN-SITU MEASUREMENT OF CHLOROPHYLL-A FLUORESCENCE

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Abstract

Eutrophication and harmful algal blooms have become an issue around the world as human land use continues to increase the amount of nutrients being released into many waterbodies. Taste and odor issues associated with many algal taxa result in complaints from consumers, increased treatment costs, and ultimately increased drinking water cost to consumers. Predicting algal blooms often requires determination of chlorophyll-a concentration, the pigment that allows algae to photosynthesize. In-vitro chlorophyll-a determination is the most accurate method, but is resource and time consumptive. In-situ methods of determining chlorophyll-a by measuring fluorescence are less intensive, but may be susceptible to environmental conditions, and therefore may not be as accurate as laboratory methods. This study evaluates the accuracy of in-situ chlorophyll-a determination in relation to in-vitro chlorophyll-a determination in the laboratory and attempts to correct any biases that may be caused by environmental conditions. Chlorophyll-a grab samples were collected monthly from May-October 2020 and analyzed under laboratory conditions. In-situ chlorophyll-a fluorescence, photosynthetically active radiation, turbidity, and qualitative lighting condition data were collected at the same location and time as chlorophyll-a grab samples. In-situ chlorophyll-a data was biased by lighting condition, resulting in underestimation of chlorophyll-a concentration by in-situ data collected under direct sunlight. A multiple linear regression model was fit using in-situ chlorophyll-a fluorescence as the response variable, and in-vitro chlorophyll-a, photosynthetically active radiation, and turbidity as predictors. The relationship between model predicted in-situ chlorophyll-a fluorescence and in-vitro chlorophyll-a concentrations was improved over initial comparisons using raw data. The procedure presented in this study represents an efficient way to increase chlorophyll-a

monitoring capacity while maintaining measurement accuracy and confidence, but should not be used as a total replacement for in-vitro chlorophyll-a determination in a laboratory.

Introduction

Eutrophication and harmful algal blooms (HABs) are a worldwide issue as anthropogenic activities continue to release excess nutrients into water sources (Prepas and Charette 2003, Gatz 2020). Many species of algae are known to produce compounds such as Geosmin and 2-Methylisoborneol (MIB), which can cause taste and odor issues in drinking water sources and require expensive water treatment upgrades to mitigate (Watson 2004). As a result, water treatment costs are drastically increased, which leads to more expense for consumers (Suffet et al. 1996). Certain algae are also able to produce different types of toxins, which can be harmful to humans and wildlife and result in water sources becoming unsuitable as a drinking water source (Sellner et al. 2003). An example of a worst-case scenario occurred in 2014 in Toledo, Ohio, when residents of the town were advised not to drink the tap water after HABs in Lake Erie resulted in microcystin in finished drinking water (Jetoo et al. 2015). Ability to predict algal blooms is critical to effectively managing drinking water sources to avoid taste and odor or complete degradation, in the worst case scenario, to a point where a source can no longer be used for drinking water. Algal biovolume is often estimated by measuring the concentration of chlorophyll-a, the pigment that allows algae to photosynthesize, in a water sample. In-vitro chlorophyll-a determination is a widely accepted method of measuring chlorophyll-a concentrations in which high-volume water samples (1-L or more) must be filtered, extracted in a solvent, and measured using either spectrometry or fluorometry (Holm-Hansen et al. 1965). Although these methods are an accurate way to determine chlorophyll-a concentrations, they are time and resource intensive, which often constrains the number of samples that can be analyzed and thus the spatial and temporal scales of most algal studies (Lorenzen 1966).

In-situ methods for measuring chlorophyll-a fluorescence have the potential to mitigate some of the resource constraints caused by in-vitro methods by allowing for measurement of chlorophyll-a fluorescence in the field (Gregor and Maršálek 2004). In-situ methods for measuring fluorescence may not be as accurate as laboratory methods, but allow for the collection of data at much finer temporal scales and across larger areas of water. In-situ measurement also allows for instantaneous assessment of potential blooms that may require immediate action where lab results may not be available quickly enough for an effective response.

In-situ fluorescence measurement may allow for collection of more data, but is not always the most reliable method and is subject to environmental phenomena that may bias measurements. One such phenomenon that is known to influence chlorophyll-a fluorescence is non-photochemical quenching, the process by which algae "quench" their fluorescence as a response to excess light energy (Muller et al. 2001). In the case of measuring in-situ chlorophyll-a fluorescence, this phenomenon may lead to underestimation of algal biovolume in a system exposed to the sun relative to an estimate derived from in-vitro chlorophyll-a analysis in a lab, where lighting conditions are kept dark. The overall goal of this study was to evaluate and correct light-related bias associated with in-situ chlorophyll-a measurement. To accomplish this goal, our objectives were 1) to collect concurrent in-vitro and in-situ chlorophyll-a data from the same locations at different times of day and year, 2) collect photosynthetically active radiation (PAR) and turbidity data consistent with time and location of chlorophyll-a data, 3) compare in-situ chlorophyll-a data with in-vitro chlorophyll-a data to look for potential biases, and 4) if biases are present, attempt correction using PAR and turbidity data in a linear regression model.

Materials and Methods

Study Site

Lake Sidney Lanier (Lake Lanier) is an 18,000-ha impoundment of the Chattahoochee river located northeast of Atlanta, GA, United States and is operated by the United States Army Corps of Engineers (figure 1). Designated uses of this reservoir include water supply, hydroelectricity, flood control, and recreation. Lake Lanier was constructed in 1956 and has since undergone extensive residential development along its shoreline. Data were collected in four different coves of the Lake Lanier that were all located in the southern part of the reservoir near the dam (figure 1).

Data Collection

Sampling took place monthly from May-October 2020 at ten sites within each of the four coves. Sites were sampled over the course of one day, and the order in which coves were sampled was randomized to increase variation in different lighting conditions sampled in each event. For in-vitro chlorophyll-a determination, 1-L of water was collected in an amber bottle from 1-m depth using a typhoon submersible pump. Samples were filtered, extracted and run on a Shimadzu spectrofluorometer following United States Environmental Protection Agency (EPA) method 445.0 for in-vitro chlorophyll-a determination (Arar and Collins 1997). A Yellow Springs Instruments (YSI) EXO 3 water quality sonde equipped with a total algae sensor was affixed to the submersible pump at the exact depth and position of the pump intake, which was used to measure chlorophyll-a fluorescence in-situ. Sonde data points were collected after a stabilization period of one minute and before water samples to avoid disturbance created by the pump. Photosynthetically active radiation (PAR) data were collected using a Li-Cor LI-193 spherical underwater quantum PAR sensor at 1-m depth. The PAR sensor was positioned as close as possible to the water sampling location, ensuring that the shadow of the boat or other sampling instrumentation did not interfere with measurements. Turbidity measurements were done with a Hach 2100Q turbidity meter using the water collected by the submersible pump. Qualitative observations of the lighting condition at each site were also recorded.

Data Analysis

The relationship between raw in-situ chlorophyll-a and in-vitro chlorophyll-a was evaluated using simple linear regression. Linear regression models were fit using pooled data and data parsed by observed lighting conditions.

The least absolute shrinkage and selection operator (LASSO) was used to evaluate variable importance and lack of correlation prior to linear modeling. The LASSO evaluates all parameters for contribution and collinearity, then shrinks the coefficients of highly correlated and less important variables to zero. LASSO was fit in the "glmnet" package (Friedman et al. 2010) version 4.0-2 in the R program for statistical computation (R Core Team 2021) using a generalized linear model with a Gaussian distribution:

$$g(Y_i) = \beta + X_{i1} + \cdots + X_{i3}$$

where $g(Y_i)$ is the expected in-situ chlorophyll-a value and X_i represents in-vitro chlorophyll-a, par, and turbidity variables. LASSO regression applies a weighting penalty (λ), found by 10-fold cross-validation, to the absolute value of the coefficient sums for standardized predictors:

$$Y_{\boldsymbol{Lasso}}(\hat{\beta}) = \sum_{i=0}^{a} (y_i - x_i\hat{\beta})^2 + \lambda \sum_{j=0}^{b} |\widehat{\beta_J}|$$

Response and predictor variables were log-transformed prior to model fitting to meet the assumptions of the Gaussian distribution.

Once important variables were identified, a multiple linear regression model was fit:

$$Y = \alpha + \beta_{x1} + \cdots + \beta_{x3}$$

where Y is the expected in-situ chlorophyll-a value and β_{xi} represents the important predictor variables identified by LASSO. Again, response and predictor variables were log-transformed to meet assumptions of the Gaussian distribution. Model predictions of in-situ chlorophyll-a were generated, back-transformed, and compared with original in-vitro chlorophyll-a concentrations using simple linear regression to evaluate the effectiveness of the model at "correcting" in-situ chlorophyll-a values.

The model was evaluated using 5-fold cross validation. Using the *createFolds* function in the "caret" package version 6.0-86 in R, the dataset was randomly divided into five equal subsets (i.e., folds). During this procedure, one of the folds was used as a validation dataset, while the other folds were used to train the model. This process was repeated five times so that each fold was used both as a training dataset and a validation dataset. Goodness of fit for each validation fold was determined by calculating the root mean squared error (RMSE). Model and cross-validation RMSE values were compared to evaluate model performance.

Results

Initial comparison of raw in-situ chlorophyll-a values with in-vitro chlorophyll-a values yielded a linear model with an adjusted coefficient of determination (R^2) of 0.65 (figure 2). When parsed by shaded, cloudy, and sunny observed light conditions, comparisons yielded linear models with adjusted R^2 values of 0.84, 0.83, and 0.68 respectively, indicating that in-situ data collected in shaded and cloudy conditions have a stronger relationship with in-vitro chlorophyll-a than in-situ data collected in sunny conditions (figure 3). It is also apparent from these data that in-situ data are underestimating chlorophyll-a concentrations when collected in both cloudy and sunny conditions (figure 3).

All three input predictor variables (in-vitro chlorophyll-a, PAR, and turbidity) were identified as important by the LASSO regression using $\lambda = 0.003$. Coefficients for in-vitro chlorophyll-a, PAR, and turbidity were $\beta = 1.026$, $\beta = -0.216$, and $\beta = 0.120$ respectively, and were all included in the final multiple linear regression model. All three predictor variables were significant in the final model ($p < 0.001$) at $\alpha = 0.05$ and the model had an adjusted R^2 of 0.83, indicating a better fit than the raw data model (figure 4). The mean five-fold cross-validation RMSE value (0.278) was comparable to the model RMSE value (0.280), further indicating that the model performs well. When parsed by shaded, cloudy, and sunny observed light conditions, comparisons of back-transformed model predicted in-situ chlorophyll-a with original in-vitro chlorophyll-a concentrations yielded linear models with adjusted R^2 values of 0.90, 0.88, and 0.95 respectively (figure 5).

Discussion

Initial comparisons of in-vitro chlorophyll-a concentrations determined in a laboratory using fluorometry with in-situ measurements of chlorophyll-a fluorescence suggest that in-situ measurements are biased based on the lighting condition under which they were measured. In-situ measurements related better with in-vitro measurements when collected under shaded or cloudy conditions than when collected under direct sunlight, which we attribute to varying levels of light intensity that were present at a site when in-situ data were collected. In-situ data collected under direct sunlight also underestimated actual chlorophyll-a concentrations, which we attribute to the non-photochemical quenching effect.

The procedure used in this study represents a relatively simple way to improve the accuracy of in-situ chlorophyll-a data collected by fluorescence sensors when fluorescence could be affected by lighting conditions. Although we examined the effect of lighting condition on in-situ chlorophyll-a fluorescence 1-m below the surface at multiple locations, the same principles most likely would apply to data collected from the same location at different depths. Rather than improved relationships in shaded or cloudy conditions, accuracy would likely improve with increased depth, as less light attenuates through the water column. Assuming a similar non-photochemical quenching effect exists in depth profiles as it does at the surface, the procedure described in this study could also be used to improve accuracy of in-situ chlorophyll-a depth profiles. Furthermore, our use of LASSO regression for variable selection ensures that our model can be applied to other datasets without overfitting issues, which is confirmed by our cross-validation results. Additionally, the data required to implement this procedure (PAR and turbidity) may already be collected by some monitoring entities or could be easily added to a monitoring regime.

As anthropogenic activities continue to adversely affect water supplies, simple and efficient monitoring procedures are becoming more essential to tracking and predicting HABs. However, accuracy should not be compromised to achieve a simpler and faster procedure. The model presented in this study would allow for more expansive monitoring of chlorophyll-a using in-situ fluorescence, while still maintaining the accuracy of expensive and time-consuming laboratory procedures that measure chlorophyll-a. While our procedure could result in reduction of laboratory chlorophyll-a analysis for many entities, we do not yet recommend complete replacement of laboratory procedures with in-situ measurements. Until in-situ sensor technology improves to a point at which it is not affected by environmental conditions, practitioners should continue to collect more accurate laboratory data for comparisons and post-calibration.

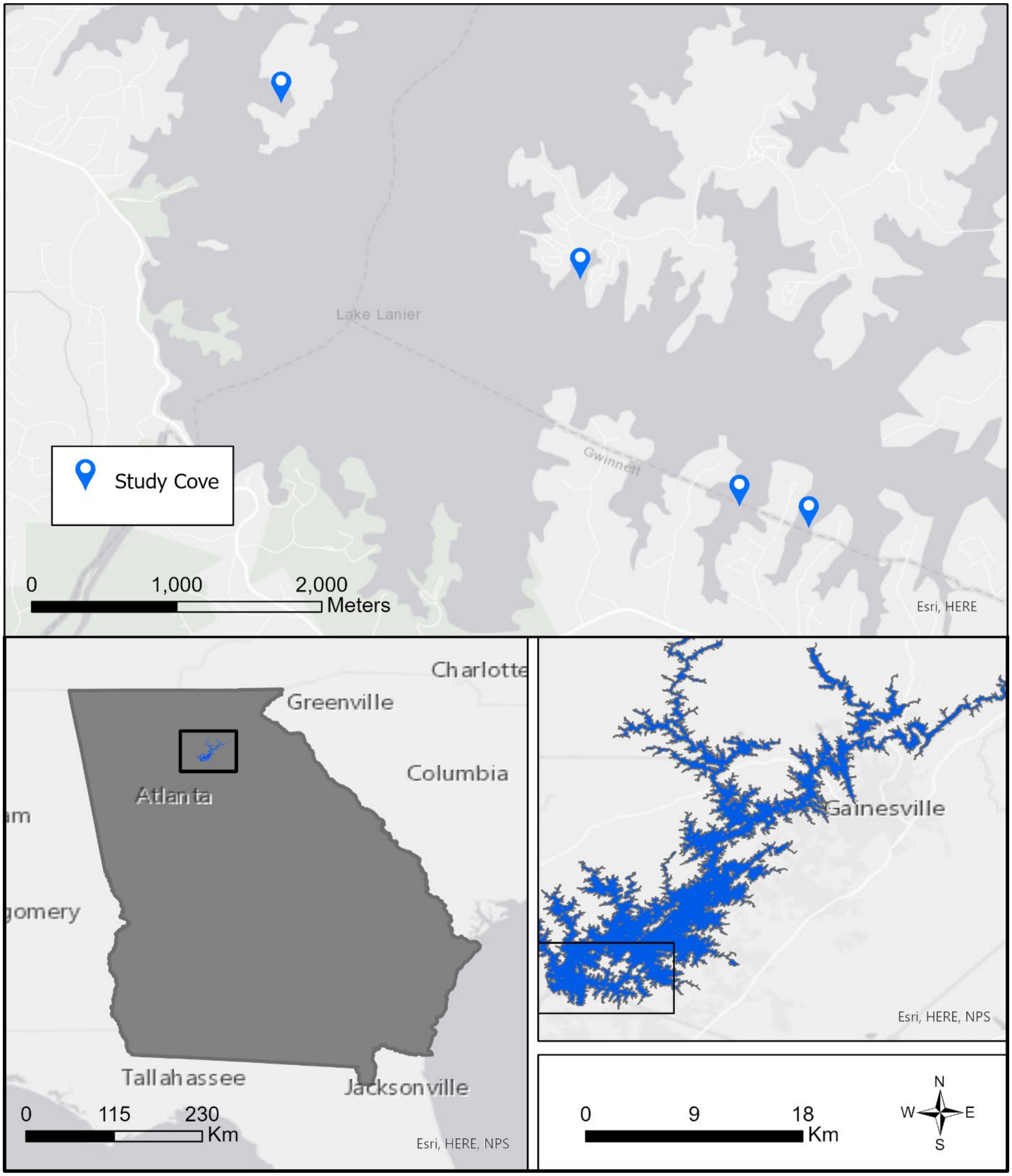

Figure 3.1. Location of the four coves from which chlorophyll-a and turbidity data were collected on Lake Lanier, Georgia and their relative locations within the entire lake.

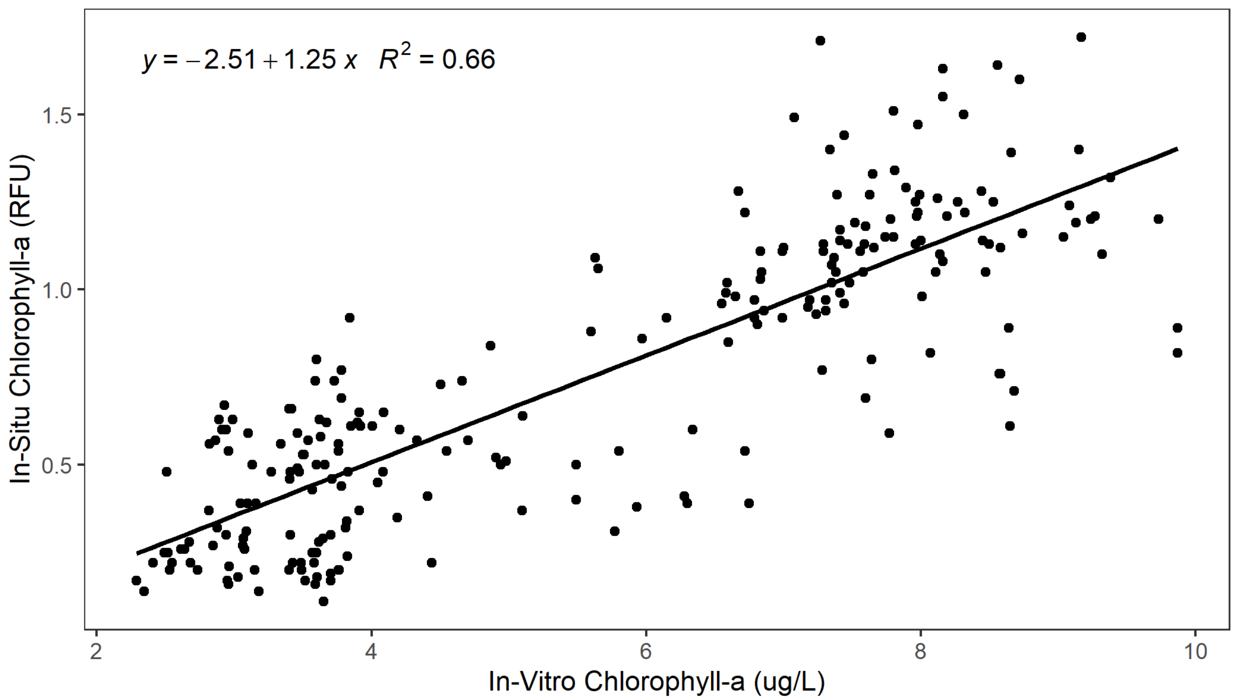

Figure 3.2. Comparison of raw in-situ chlorophyll-a fluorescence and in-vitro chlorophyll-a concentration data that were collected from May-October 2020 from 40 different sites within four coves in Lake Lanier, Georgia. The regression equation and adjusted R^2 value were determined using simple linear regression on log-transformed values.

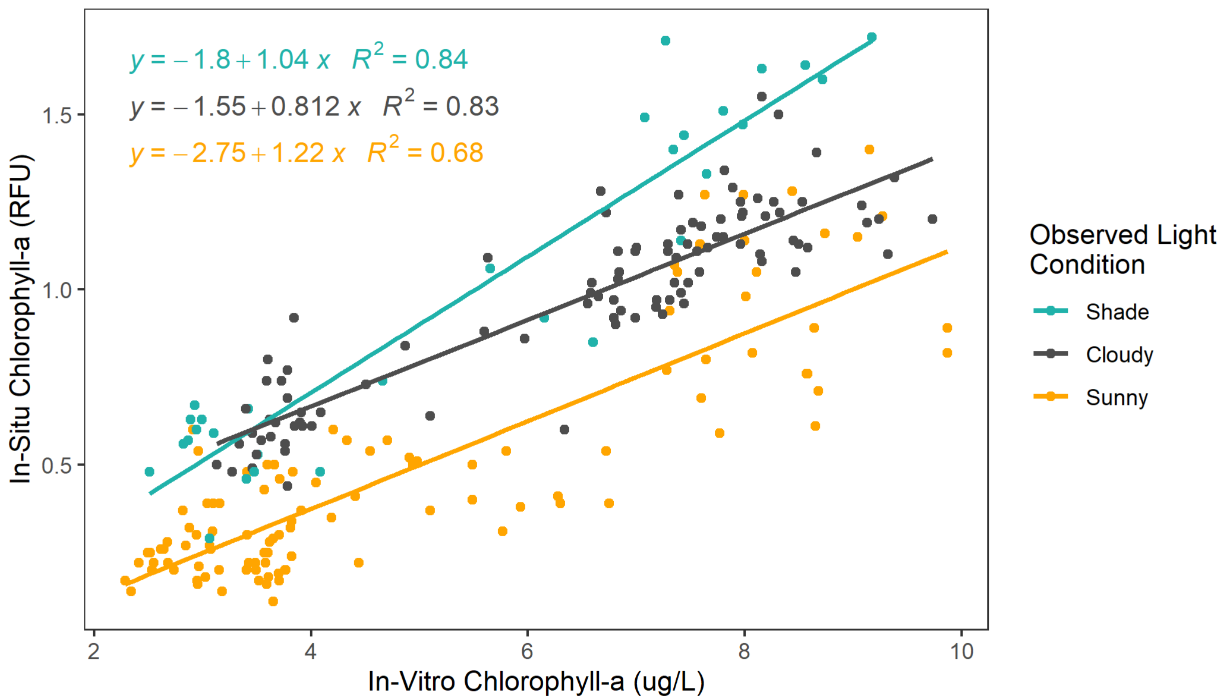

Figure 3.3. Comparisons of raw in-situ chlorophyll-a fluorescence and in-vitro chlorophyll-a concentration data parsed by qualitative lighting observations that were collected from May-October 2020 from 40 different sites within four coves in Lake Lanier, Georgia. Regression equations and adjusted R^2 values were determined using simple linear regression on log-transformed values.

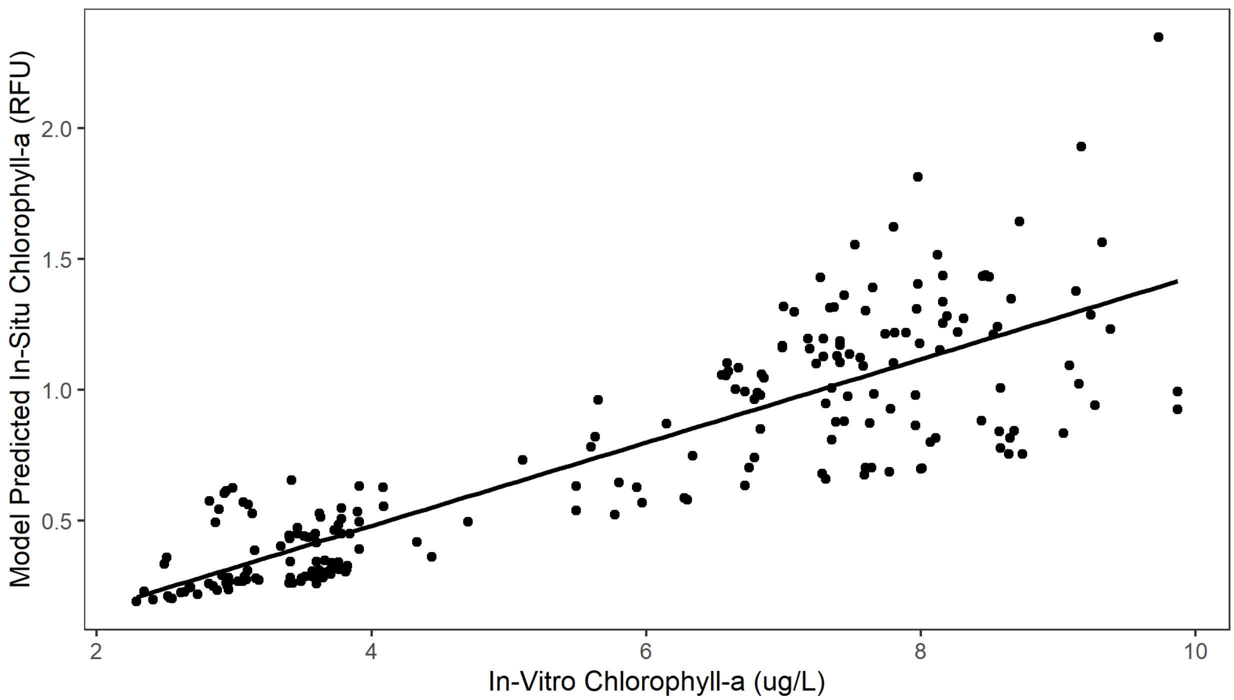

Figure 3.4. Comparison of back-transformed model predicted chlorophyll-a fluorescence and raw in-vitro chlorophyll-a concentration data that were collected from May-October 2020 from 40 different sites within four coves in Lake Lanier, Georgia.

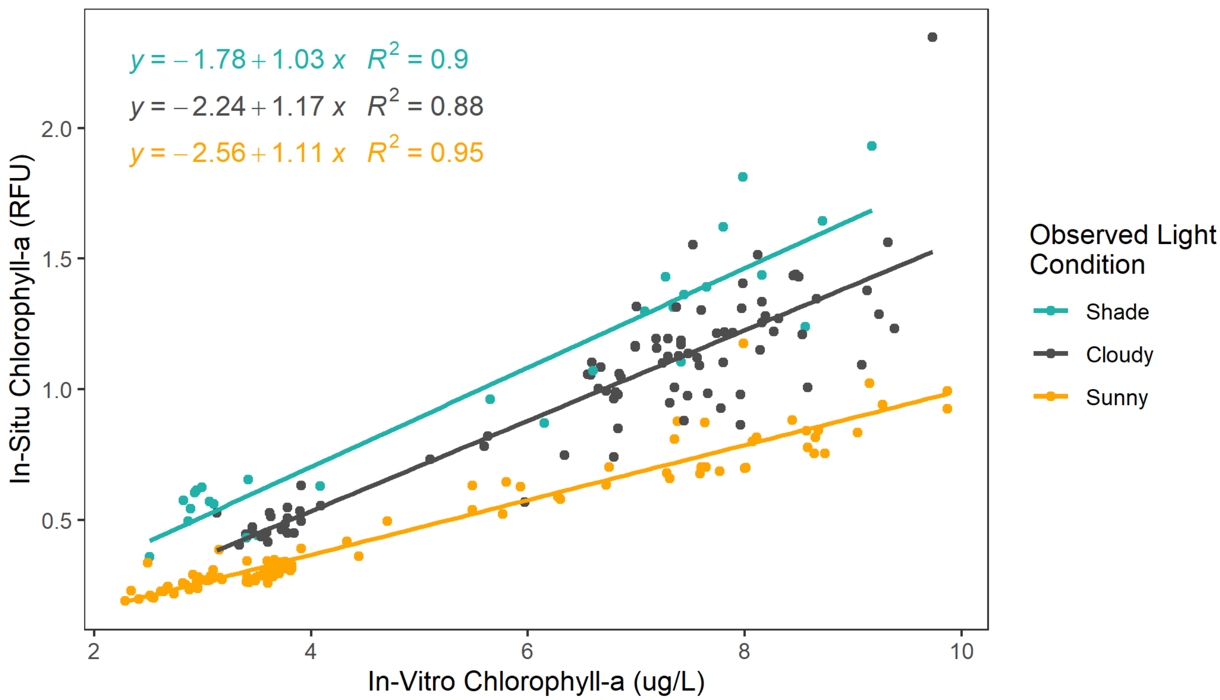

Figure 3.5. Comparison of back-transformed model predicted chlorophyll-a fluorescence and raw in-vitro chlorophyll-a concentration data parsed by qualitative light condition observations that were collected from May-October 2020 from 40 different sites within four coves in Lake Lanier, Georgia. Regression equations and adjusted R^2 values were determined using simple linear regression on log-transformed values.

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CHAPTER 4

GENERAL CONCLUSIONS

Effects of Onsite Wastewater Treatment

We were unable to attribute variations in shoreline water quality at our study sites to presence or placement of onsite wastewater treatment (OWT) systems. OWT systems are designed for effective treatment of wastewater, and our results suggest that the systems evaluated as a part of our study are performing as they should. Although we are unsure whether the systems within our study coves were installed following best practices or whether best maintenance practices are being done, there was no measurable effect in the shoreline water we sampled. However, we can conclude that residential development has an effect on water quality based on the significant difference found between E. coli concentrations in developed and undeveloped coves. Effects of residential development appear to be related to surface runoff and stormwater inputs, shown by the significant relationship between precipitation and all five water chemistry parameters we measured. Potential non-point source pollutants from residential areas could include animal (pet or wildlife) feces or fertilizers used on lawns and gardens. Future research in this area should include a DNA tracer study, which would distinguish human E. coli bacteria from animal E. coli bacteria. Presence of human E. coli bacteria could then be attributed to septic tank leakage. Management in densely developed reservoir systems should focus on limiting non-point source inputs from surface runoff and stormwater by limiting fertilizers and animal waste at nearshore homes.

Chlorophyll-a Monitoring

We present an effective method for improving accuracy of in-situ chlorophyll-a fluorescence measurements collected under varying light conditions, whether in the sun or shade or at the surface or deep. This method is implemented using data that are either already collected as a part of most monitoring regimes or could be easily collected without the addition of any rigorous field or laboratory procedures. We recommend this method not as a total replacement for in-vitro chlorophyll-a determination under laboratory conditions, but as a way to collect additional chlorophyll-a data in-situ without additional laboratory analysis and to increase confidence in in-situ data. In resource-limited situations, in-situ data could be collected on a much larger spatial scale and could then be validated by comparing with fewer in-vitro measurements and using our method to correct for light-related biases. Future research could potentially investigate the remaining bias that is not corrected by our model, as model predicted in-situ values taken under sunny conditions still underestimated the in-vitro chlorophyll-a concentrations. However, care should be taken to retain simplicity and ease of implementation for any future models. Any additional parameters may cause model overfitting, and should be checked for contribution and collinearity using the least absolute shrinkage and selection operator prior to their addition.