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Effects Of Nutrient Overload And Environmental Conditions On Algal Bloom Formation: A Case Study Of Water Sources In Eastern Kentucky

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EFFECTS OF NUTRIENT OVERLOAD AND ENVIRONMENTAL CONDITIONS ON ALGAL BLOOM FORMATION: A CASE STUDY OF WATER SOURCES IN EASTERN KENTUCKY

BY

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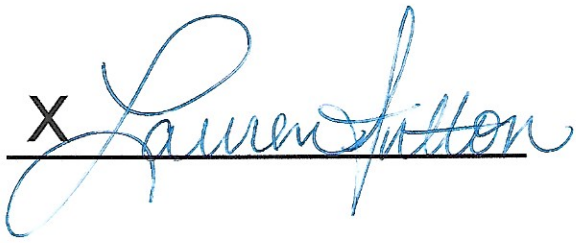
X 

Date: 4/8/2020

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BY

LAUREN NICOLE SUTTON

Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

2020

DEDICATION

This thesis is dedicated to my loving parents Lucinda Sutton and Matthew Graves.

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I would like to acknowledge my advisor, Dr. Cindy Tran, for giving me insurmountable support and encouragement through this project. I couldn't have attained the growth as a scientist without my advisor and committee members (Dr. Judy Jenkins, Dr. Karim Abdelhay, and Dr. Alice Jones). I would like to thank the undergraduates who may have been helpful on this project. I am very grateful for the help from the Chemistry department and Natural Areas department within Eastern Kentucky University for the use of instrumentation. I would also like to thank Andy Thompkins of Danville Water Plant for help with this project and donation of instrumentation. This project was funded by Kentucky Water Resources Research Institute (KWRRI). I would like to thank my parents Lucinda Sutton and Matthew Graves. Not only did they support me in my endeavor of continued education in graduate school, they supported my decisions even when I felt like I couldn't continue. To those who feel like giving up, continue to push through to accomplish your goals and prove those who doubt you wrong.

ABSTRACT

Eastern Kentucky is home to many private ponds used for agricultural and recreational purposes. Each year, the owners of these ponds observe harmful algal blooms (HABs) that release toxins into the water, potentially limiting the use of these ponds. A pilot study in summer 2018 observed a harmful algal bloom (HAB) occurring in one pond in Madison Country that roughly correlated with a rise in water sulfate levels with no detectable levels of nitrate or phosphate present. A follow-up study of this pond and others in the area was conducted during Summer 2019. The purpose of this project was three-fold: 1) compare findings reported in literature for larger water sources to Kentucky water sources, 2) to identify trends in water nutrient levels and environmental conditions that would indicate the imminent formation of HABs in these water sources and 3) to determine if commercially-available test kits would provide adequate information for a lay person to monitor their private water sources and predict HAB formation.

To accomplish these aims, the nutrient levels and environmental conditions of 10 different water sources were monitored throughout summer 2019. Taxonomical characteristics identified the algae strain(s) present in potential HABs and nutrient level quantification was achieved using a combination of EPA-validated laboratory techniques for major anions (ion chromatography, EPA method 300), phosphates (spectrophotometry, EPA method 365.3), and turbidimetry (EPA method 180). Commercially available semi-quantitative kits from LaMotte and HACH were utilized as secondary measures of phosphates, nitrates, nitrites, and sulfates. Environmental conditions such as rainfall, water pH, dissolved oxygen, conductivity and temperature

were also monitored. From this work, it was found that sulfate levels were not correlated HAB formation and confirmed that HABs can form in these water sources with nitrate and phosphate levels below detectable limits. While turbidity and dissolved oxygen levels were correlated with HAB formation, these are not causative of the HAB. Data mining using principal component analysis demonstrated the correlative relationships of tested variables and observed trends, though no conditions were shown to be clearly causative of HAB formation in these sources.

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1.1 Overview

Harmful algal blooms (HABs) are defined as the formation of toxin-producing algae in water sources such as ponds, lakes, rivers, oceans, and reservoirs. These blooms release harmful toxins into the water which can lead to drastic changes to the aquatic ecosystem.[1] Major changes from algal bloom exposure can result in unsustainable conditions for aquatic wildlife.[1] Research into the identification of the underlying causes of HABs has focused primarily on major water sources and identified an influx of nutrients such as nitrates and phosphates as the primary factor in the promotion of algal growth.[2] As HABs can occur in smaller bodies of water and hurt the environment and economies at a local level, this research examines the environmental conditions and nutrient levels in smaller water sources that could lead to predicting bloom formation.

1.1.1 Algae

Algae are chlorophyll-containing organisms that can be either single celled or multicellular depending on the species including types such as green algae, red algae, and brown algae.[1] Algae can be microscopic in size, such as phytoplankton, or macroscopic, such as seaweed and kelp. These photoautotrophic organisms use available chlorophyll to absorb sunlight which is converted into energy through photosynthesis. Algae is considered the most abundant oxygen producer of any plant.[1] When an overabundance of algae occurs, it forms an algal bloom.[2] These formations result in large spreads of algae. These blooms can vary in shape, size, pigmentation, and in the ratios of aquatic organisms responsible for formation. Nonharmful algae includes

species such as Chara/Muck grass, Filamentous, Duckweed, and Spirogyra/Pond Scum (Figure 1.1 A-D). Nonharmful algae are visually more vibrant colored, though dexterity can't be used to easily distinguish harmful from not. Strong odor is associated with harmful algae species presence as well typically as a fishy, sulfur stench. Conditions visually different from normal raise suspicion to evaluate the health of a water source. Field observation guides are used to assess potential algae overabundance resulting in distorted or vibrant colors, not associated with normal conditions of the water source. Visual observations don't serve as a confirmation of harmful species, further assessment is required in the lab to determine presence.

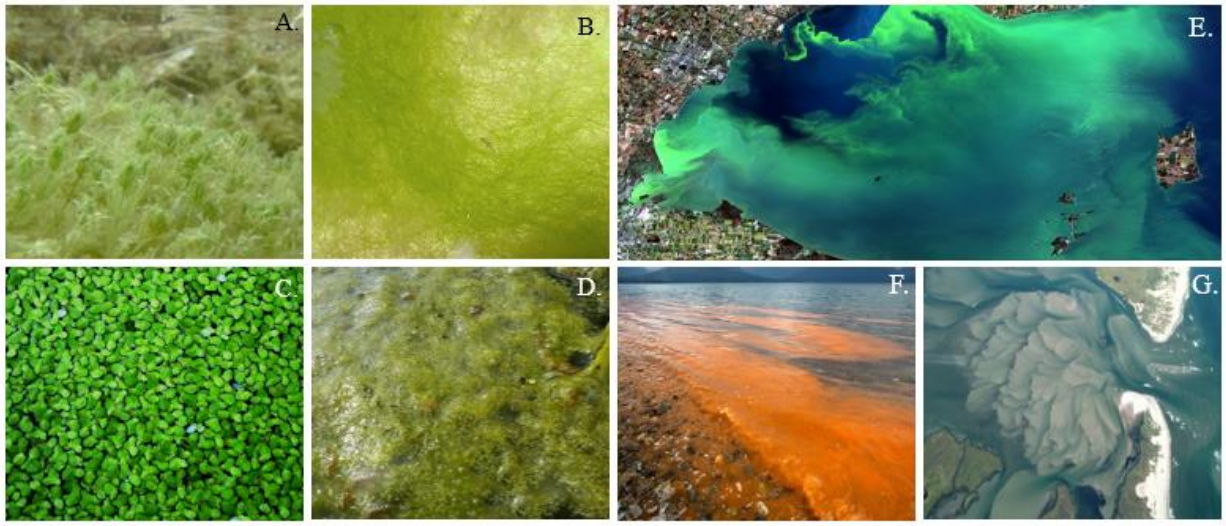

Figure 1.1. Variety of algae species which can be present in water sources including nonharmful and harmful species. Nonharmful species include A) Chara/Muck grass, B) Filamentous, C) Duckweed, and D) Spirogyra/Pond Scum. Harmful species include E) Cyanobacteria, F) Red Tide, and G) Brown tide.

Harmful algae can often be distinctly spotted when vibrant colors, overly thick or thin viscosity texture, and foul odors are present near the water source. Harmful algae blooms include species such as blue green (Cyanobacteria), red tides (K. brevis)[3], and brown tides (A. anophagefferens)[4] (Figure 1.1 E-G). Blooms vary in visible

characteristics including size, shape, color, and turbidity. Different species have a variety of characteristics associated with present microorganisms. Growth conditions are affected by factors of availability of sunlight, carbon dioxide, and nutrients such as phosphorous and nitrogen. Additional environmental conditions can alter the life cycle of algae including changes in temperature, pH, and salinity within the aquatic environment. Eutrophication, or an excess of nutrients in an environment, is reported as a leading cause of the growth of algal blooms.[2] In addition to eutrophication, stagnant water flow, high temperature and pH, and low turbulence are also reported to play a role in the rapid formation of blooms.[1] In freshwater sources, blue-green algae (cyanobacteria) is the primary species in HABs.[5]

During the formation of blooms, toxin-producing organisms create unstable conditions that not only affect aquatic plant life but also human and animal health. Secondary metabolites within cyanobacteria are responsible for production of toxins including hepatotoxins, neurotoxins, and dermatoxins.[1] Exposure to Hepatotoxins including Microcystin and Nodularin primarily result in liver damage although other health effects range from acute to severe depending on exposure to toxin type and may include severe gastrointestinal damage, heart failure, or death.[1]

1.1.2. Indicators of blooms

When assessing the presence of an algal bloom, the conditions that led up to this event should be evaluated to understand the requirements for algae growth. Literature studies have shown parameters that must be met to indicate bloom growth including stagnant flow, low dissolved oxygen, high phytoplankton, excess of nutrients and high temperature.[6,7] However, establishing clear indicators during formation events can

prove to be difficult. The guidelines that are used for bloom observation are vague and need clarification to establish if such variables are applied to water sources of all sizes and use. It is understood that environmental conditions and nutrient loads play some type of role in overabundance of phytoplankton resulting in bloom formation.[5-7]

Prior research in the field has established general guidelines of present indicators during bloom events. These guidelines establish favorable conditions for bloom formation including optimal temperatures reaching at 25°C, low dissolved oxygen values and excess of nutrient loads. There is no one required value for dissolved oxygen that must be met before bloom formation.[8] These guidelines do not include quantified amounts of nutrient loads required for overabundance growth of algae that need to be prevented. Rather there are guidelines that suggest water bodies should contain 20 μg/L of phosphorous to restrict overabundance growth of algae.[9] However, the World Health Organization (WHO) studied water sources with phosphorous concentrations exceeding 100 μg/L.[9] This amount did not trigger the low health alert associated with cyanobacteria presence. Having higher concentrations of phosphorous without a trigger warning from WHO led to understanding other factors were involved in the development of blooms.[9] However, these studies utilized large bodies of water such as Lake Eerie,[10] the Gulf of Mexico,[6] or the Everglades[11] to draw their conclusions and it is unclear if these guidelines would apply similarly to smaller water sources.

Bloom research has been focused on populous areas near the Great Lakes where the drinking water source is relied on by approximately 30 million people.[12] This area is known for being affected by agricultural and industrial run off as well as occurrence of large harmful algal blooms. Forecasting technology is used within the area to educate

the public about bloom detection based on geographic images. This monitoring system uses real time imagery to locate, monitor, and quantify algal blooms in coastal and lake regions in the United States.[13]

1.1.3. Prior Research

Research in this field focuses on water sources that are primarily larger, man-made resources that may be the source for drinking water for populations. Water sources monitored by state or federal institutions follow guidelines established by the Clean Water Act which enables quality regulations for pollutants and contaminant presence in water sources including lakes, rivers, streams, wetlands, and coastal areas across the nation.[14] This act addresses chemical, physical, and biological standards that are to be indicative, while recognizing state and federal responsibility of pollution and improvement to treatment facilities when necessary. The EPA is conducting research to determine approachable ways to reduce nutrient loadings in water sources, monitoring and sensing bloom formations, toxicology of cyanobacteria, and further understanding the effects from exposure to harmful algal blooms.[15] Predictive modeling has been used in multiple projects focused on bloom formation to understand the chemical and biological indicators that occur during formation. United States Geological Survey monitor nutrient concentrations and loads in water sources across the nation using optical sensor technology. Nutrient variability is assessed using models to advance knowledge of nutrient sources and identifying bloom formation.[16] Other aspects of research regarding algal blooms included topics such as the spread of toxins through lake spray aerosol and persistence through drinking water processes and controlled growth studies for mass production of algae strains used for biofuel development.[17,18]

Growth control research has been used to understand what parameters are necessary for HAB formation. Production of algae and the capabilities of have been studied for development of biofuel. Companies like ExxonMobil have focused on reducing the carbon footprint on the environment through production of biofuels with materials like algae.[19] Though this research is based on specific strains of algae to produce oil, this process uses algae to convert into the biomass into fuel for engines.[18] Parameters of interest include availability of sunlight and excess loading of nutrients, with the intent to extrapolate certain species of algae to promote growth of algae for mass production of biofuel purposes. The experimental design focuses on different techniques for establishing the more efficient and feasible way to growth algae on a mass scale. Parameters required for optimal growth of algae is specific to the algae species of interest. For algae growth approximately 10 hours of sunlight per day is needed for optimal growth conditions not exceeding 18 hours.[20] Some loss in biomass is expected due to natural processes of respiration during the night hours.[21] Optimal temperature required for algae growth included temperature between 15°C to 30°C including irritation of algae culture.[21] This allows for aeration of the culture and exposure of all algae to more sunlight. Biomass growth of algae is specific to the algae strain of interest including the nutrient culture that is required as well. In this prior research with ExxonMobil, the strains of interest include those that produce the most amount of bio-oils. This includes the strain *Nannochloropsis gaditana* which requires a growth media with depleted nitrogen.[22] The medium environment that is used for growth of this algae culture includes Guillard's F/2 medium which contains a variety of different nutrients including approximately 75 ppm (63.6%) sodium nitrate ($NaNO_3$), 5

ppm (4.2%) sodium phosphate monobasic monohydrate($NaH_2PO_4 \cdot H_2O$), 30 ppm (25.5%) sodium metasilicate nonahydrate($Na_2SiO_3.9H_2O$), 4.36 ppm (3.7%) EDTA disodium salt(Na_2EDTA) and additional smaller nutrients.[23]

1.2. Project Objectives

The specific goals of this work are as follows:

1. Compare findings reported in literature for larger water sources[6,10,11] to Kentucky water sources.

2. Identify trends in water nutrient levels and environmental conditions that indicate imminent formation of HABs.

3. Determine if commercially available test kits provide adequate information for non-scientists to monitor their water sources.

1.3. Instrumentation Theory

1.3.1. Ion-exchange chromatography

Ion-exchange chromatography is a separation technique that separates ions based on charge, size, and affinity of the ion for a charged resin. The extent of separation of ions stems from the relative amounts of time each ion spends adsorbed to the surface of the resin rather than moving past the resin in the mobile phase. This balance of time is dictated by the equilibrium relationship of the ion on a resin and in the mobile phase. Here, this technique will be used to quantify the level of anionic nutrients present in the water samples. The basic instrumentation for this technique requires several parts, outlined in Figure 1.2.

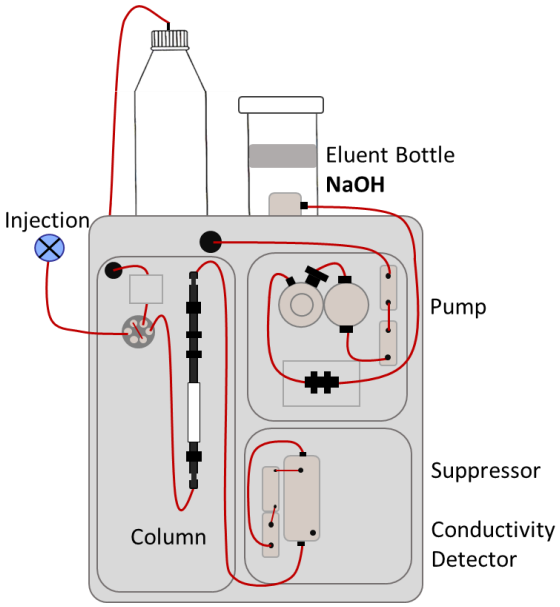

Figure 1.2. Schematic of Thermo Scientific Dionex Integrion HPIC system used in this project.[24]

In ion chromatography, the injected sample is carried through the instrument by a buffered solution or mobile phase.[25] The mobile phase is a pH-controlled aqueous solution; in the case of anion exchange, a basic solution is used. The stationary phase is a charged resin; in the case of anion exchange, the surface is functionalized with quaternary amine groups (NR^{+}_{4}) stabilized by negatively charged hydroxide counter ions.[24] As the anions of interest flow through the column, they displace the hydroxide counter ions and adsorb to the surface of the resin. As the mobile phase keeps flowing through the column, the excess hydroxide ions will dislodge the anions from the sample and the anions will continue to move down the column until they interact with the resin again.

Anions that are larger in size and/or more highly charged have stronger affinity for the column and will require more interactions with the counter ions to be desorbed from the resin surface, ultimately spending more time adsorbed to the resin than moving

through the column. This will cause the larger, more highly charged anions to elute or exit the column later in time than those with smaller size or charge. As the adsorption of an anion on the resin is an equilibrium process, it can be said that fluorine ions, with small size and low charge, have more of an affinity for the mobile phase while sulfate ions have a stronger affinity for the resin. The effect of these varied equilibria on elution time, or the time it takes for an anion to exit the column, can be seen in Figure 1.3.

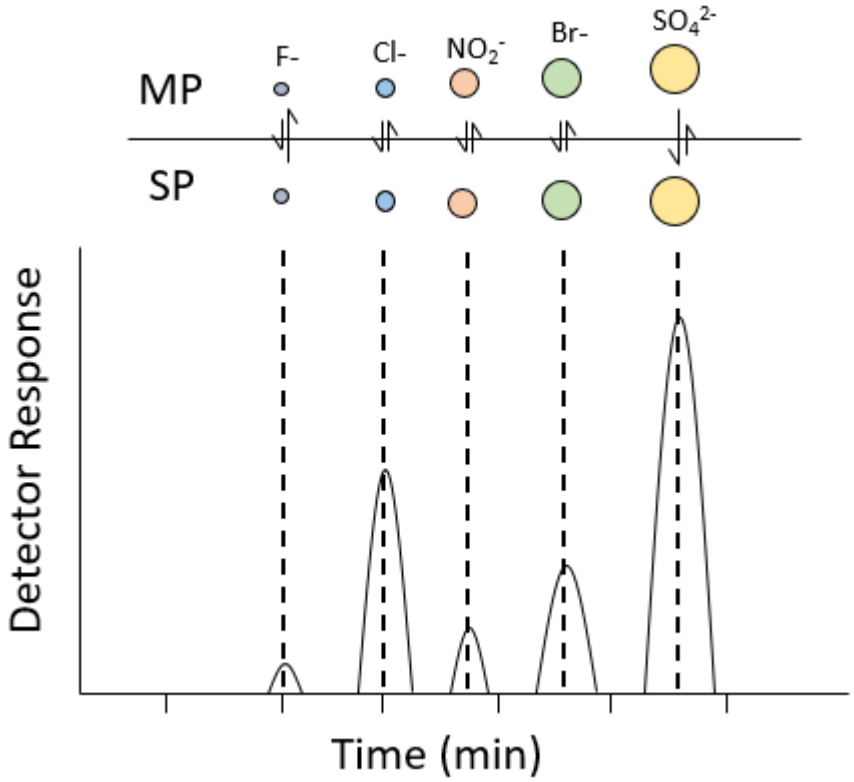

Figure 1.3 Example of ion exchange interaction inside column between anion and resin ions (top) resulting in a chromatogram (bottom).

Many of the analyte anions studies here are the conjugate bases of a weak acid. The conversion between the acid and conjugate base forms are also described by an equilibrium expression where the acid dissociation constant, K_a, describes the relationship between the two forms. When the mobile phase pH is lower than the

analyte's pKa, the analyte is more likely to be protonated than unprotonated, leading to a faster elution time due to the weakening of the anion-resin interactions (Figure 1.4 B). If the pH is higher than the pKa, the anions are more likely to be deprotonated and will elute slower as the position has shifted the opposite direction. Increasing the basicity of the mobile phase results in stronger interactions between the anion and resin and longer elution times (Figure 1.4 A).[28] Thus, controlling the pH of the mobile phase by using a buffered solution that resists changes to pH with the addition of anions from the sample minimizes any internal variance in separation efficiency within the column.

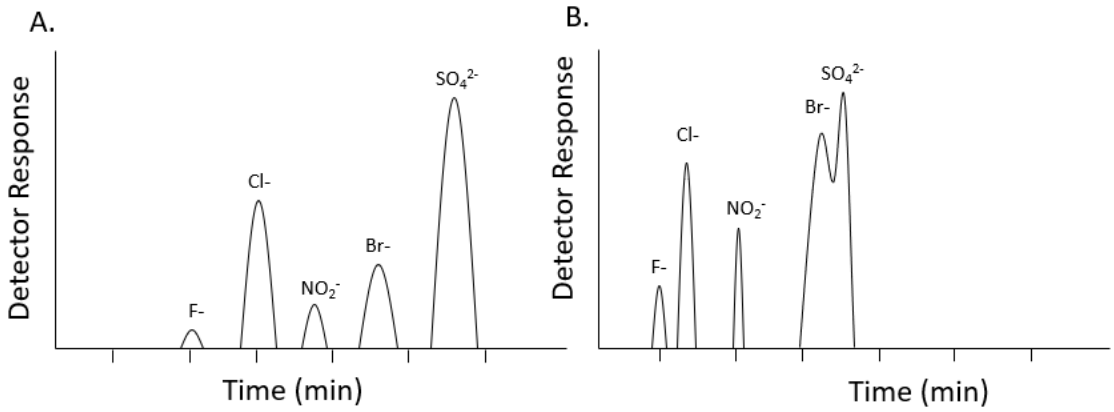

Figure 1.4 Example of chromatograms with mobile phases at A) high pH and B) low pH.

In addition to mobile phase pH, anion elution times are also affected by the concentration of analytes and column temperature so these aspects must also be controlled throughout the separation.[26] The addition of a high ion concentration of competing ions, such as in the introduction of the sample, shifts the equilibrium position to elute solute ions faster. This disruption is due to weakened interactions between the anion and resin resulting in counter ions of stationary phase being displaced easier. [28] Similarly, altering the column temperature increases the exchange rate of sample anions

between the mobile phase and the resin, altering retention times and ion selectivity.[29] Selectivity of ions increases with temperature for smaller ions with a shorter retention time. With decreased temperature, selectivity decreases with larger ions, extending the retention time.[30] To avoid any variations in elution time between samples, the temperature of the column is held constant.

As ions are eluted from the column, they are detected by monitoring the change in solution conductivity, and the resulting ion levels are displayed in a chromatogram. Known standards of the anions of interest are analyzed prior to samples of unknown composition to establish the elution order of the analytes exiting the column (Figure 1.5 A). Since the conductivity measured is directly proportional to the concentration of anions present in the eluting solution, a series of standards composed of the known analytes at various concentrations can be used to calibrate the method and form a calibration curve (Figure 1.5 B). The best fit line for the plot of standard concentration vs. signal (using peak area) can be used with the measured signal of an unknown to quantify the concentration of the analyte in the sample.

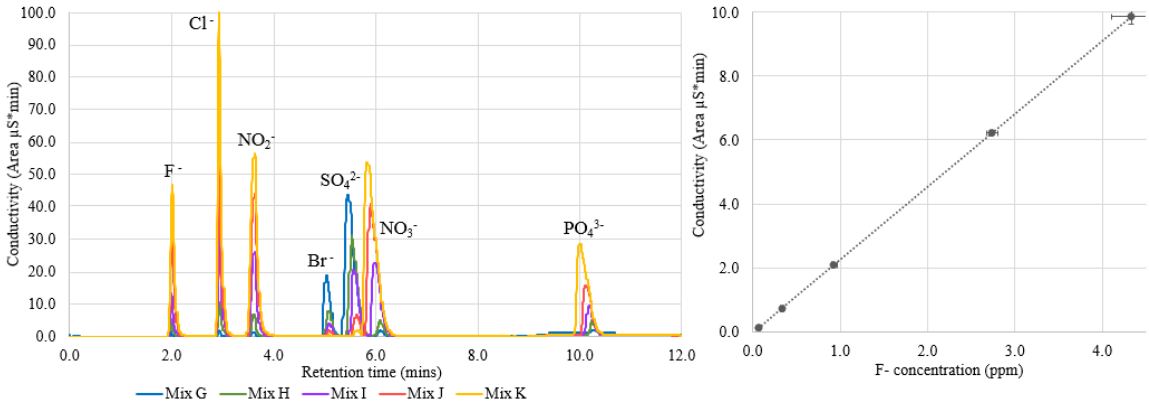

Figure 1.5 A) Chromatogram of calibration standard mixes containing a range of ions at various concentrations and B) the resulting calibration curve for the fluoride anion eluting at approximately 2.0 minutes.

Anions expected to be observed within water samples included fluoride, chloride, nitrite, bromide, sulfate, nitrate, and orthophosphate based on applied methodology from EPA guidelines for evaluation of anions.[31] The EPA guidelines for acceptable amounts of anions apply only to drinking water sources, so anion levels in private water sources are not monitored. Anions in water sources are attributed to varied origins.[31] Though levels of bromide are naturally quite low in the environment, heightened values are found in areas affected by types of shale, fossil fuels, coastal soil, or seaborne aerosols.[32] Chloride presence can be attributed to agricultural and industrial runoff, wastewater from treatment facilities, road salt, and rocks high in chlorides.[31] In low concentrations(ppm), chloride helps sustain suitable conditions for aquatic and plant wildlife. Low levels of fluoride can be found originating from rocks high in fluoride, wastewater from treatment facilities, or corrosion from pipes.[33] Fluoride concentrations are naturally low(< 0.1 ppm)[34] in water as higher concentrations can harm aquatic wildlife.[33] Treatment facilities add chloride and fluoride to the water supply in the treatment process for drinking water sources for a variety of health benefits.[31]

Analytical techniques used in this project focus on inorganic forms of nitrogen as these play a large role in HAB development. These inorganic forms can exist in the free state such as nitrate, nitrite, and as a gas.[31] Nitrate and nitrite ions are expected to be present in water sources as they are utilized through a recycling processes by animal and plant life.[35] These forms are present in water originating from wastewater contamination and municipal, industrial runoff, or agricultural runoff. High concentrations of nitrate(0.1-10 ppm)[8] have been attributed as a leading cause of

eutrophication where nutrient abundance promotes growth of algae and phytoplankton.[36]

Phosphate can take a variety of forms in water samples including inorganic and organic. The form of interest for this study is the inorganic form orthophosphate (PO_4^{3-}) as it contains a natural presence in water and sewage. Orthophosphate forms can be found in other species dependent on pKa and pH of buffer solution which include forms H_3PO_4, $H_2PO_4^-$ and HPO_4^{2-} that can be present.[37] The exact form of orthophosphate is based on the equilibrium between the forms and is controlled by the surrounding pH. In the high pH of the IC mobile phase, the unprotonated form of orthophosphate dominates and is the form being analyzed using ion chromatography. Other forms of phosphorous present in water sources include organic forms such as elemental P which can be found naturally in minerals or rocks and within the breakdown process of pesticides. As algae decays, the organic form of phosphorous is converted to the inorganic form by decomposition and bacterial presence.[38] Low concentrations of phosphates between 0.025 mg/L to 0.1 mg/L are necessary for sustaining aquatic plant and wildlife and in those concentrations, it is not harmful to human or animal health.[39] Increases in concentration can negatively affect the ecosystem due to over stimulation of algae and phytoplankton growth.[38]

Sulfates can originate from rocks containing minerals, wastewater from sewage treatment plants, and runoff from municipal, industrial, and agricultural sources.[39] Lower concentrations(< 0.5ppm)[38] of sulfate can inhibit algae growth while an oversupply of sulfate can result in harmful health affects to animals. Sulfate presence is essential in algae cell development as it is reduced to sulfide which is critical for cell

development. Sulfate is assimilated and stored after uptake in the cytoplasm or vacuole. Through ATP reduction, sulfate is reduced to sulfite, where the form is converted to sulfide by an enzyme known as sulfite reductase. This enzyme uses sulfide through assimilation to create amino acids such as cysteine.[40]

1.3.2. Spectrophotometry

Spectrophotometry involves the quantitative measurement of interactions between molecules and electromagnetic radiation. Molecules absorb energy in the form of light (photons) while light passes through the sample. Energy absorbed in the visible or ultraviolet wavelengths may provide electrons that are excited to higher energy levels.

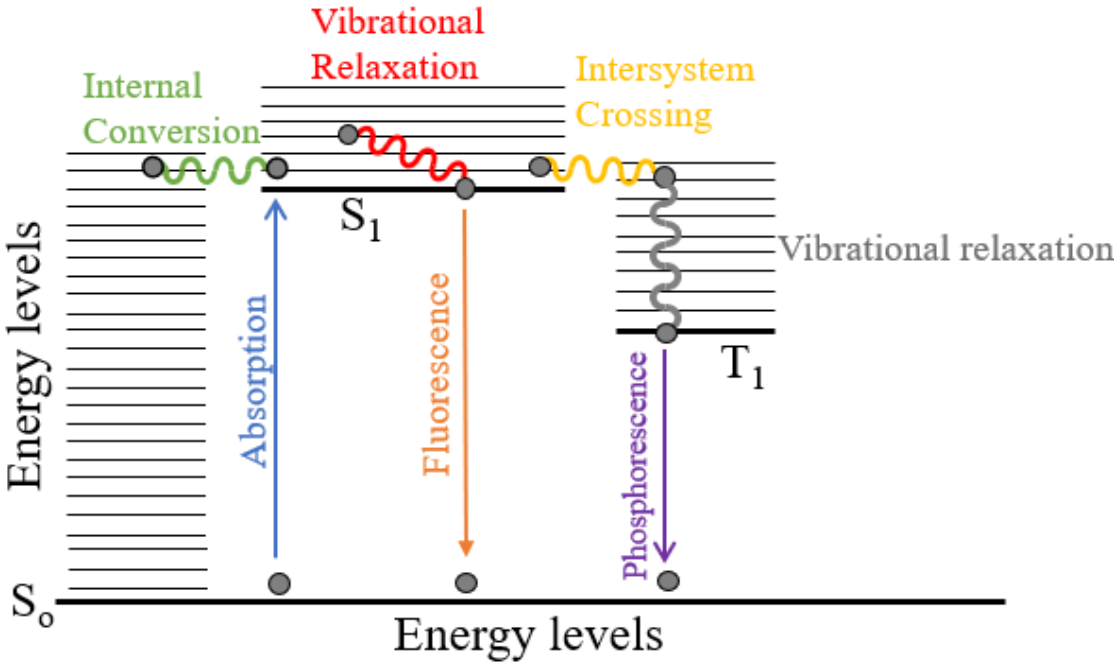

Figure 1.6 Jablonski diagram representing molecular energy transitions.

An energy diagram, known as a Jablonski diagram, illustrates the processes a molecule can go through when experiencing excitation from a ground state (S_0) due to

exposure to electromagnetic radiation.[41] Excitation involves the molecule absorbing a 1 or more photons of light and being excited to a singlet state. Once excited, there are several options for relaxation. The energy can distribute across the bonds present through vibrational relaxation without emitting another photon of energy (Figure 1.6). The molecule can relax back to the ground state by either fluorescence or phosphorescence.[41] In both cases, there is an emission of light in the form of a photon; the difference between fluorescence and phosphorescence, respectively, stems from if the molecule is in a singlet excited state (S_1) or triplet excited state (T_1) (Figure 1.6). The ability of a molecule to absorb energy at specific wavelengths depends on the energy required to make the electronic excitation transition to the excited state and will vary by molecule.[42] However, multiple analytes can be absorbed at a specific wavelength which can interfere with accurate measurements of analyte concentration. Other interferences can be seen as light scattering being interpreted as absorbance of an analyte.[42]

A basic spectrophotometer includes a light source, monochromator, and a detector. The light is directed through a monochromator which serves as a wavelength separator to isolate the wavelength of interest for the analysis. As shown in Figure 1.5, the monochromator in the instrument used in this work includes a pair of slits and a diffraction grating. The diffraction grating splits polychromatic light into its component wavelengths so only the wavelength of interest is passed through the exit slit.[43] The spectrophotometer in Figure 1.5 is classified as a double beam which splits the light exiting the diffraction grating into two beams, directing light through a reference sample in addition to the sample of interest. The dual detectors measure the intensities

of the light the is transmitted through the reference and the sample. This set up allows for more accurate measurements as it simultaneously measures the ratio of light intensity between the sample and reference.

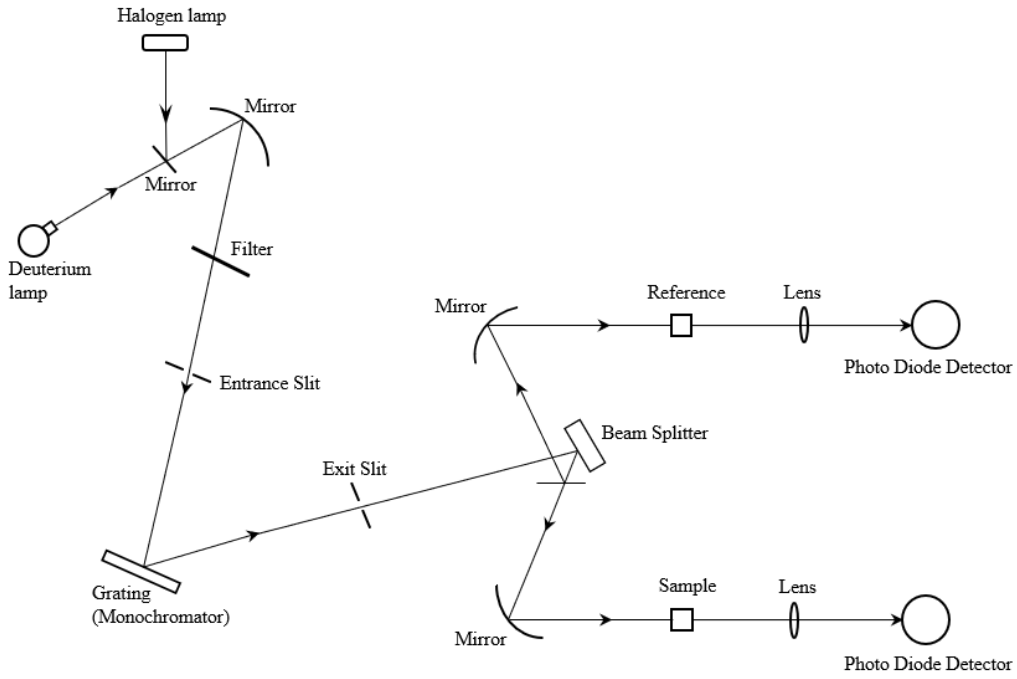

Figure 1.7 Schematic of Perkin Elmer Lambda 35 Spectrophotometer system used in this project.[44]

The detector directly measures the amount of light transmitted and absorbance is then calculated according to Equation 1.1 as given by Beer Law. The absorbance of a solution is directly proportionate to concentration (c), the path length of the light through sample (b), and the molar absorptivity of a species (ε). Samples of known concentration mixes can be used to establish the linear relationship between concentration and absorbance which can be used to determine the concentration of the sample. This technique was utilized using a method for quantification of phosphate concentrations.

$$A = \varepsilon bc \qquad \text{Equation 1.1}$$

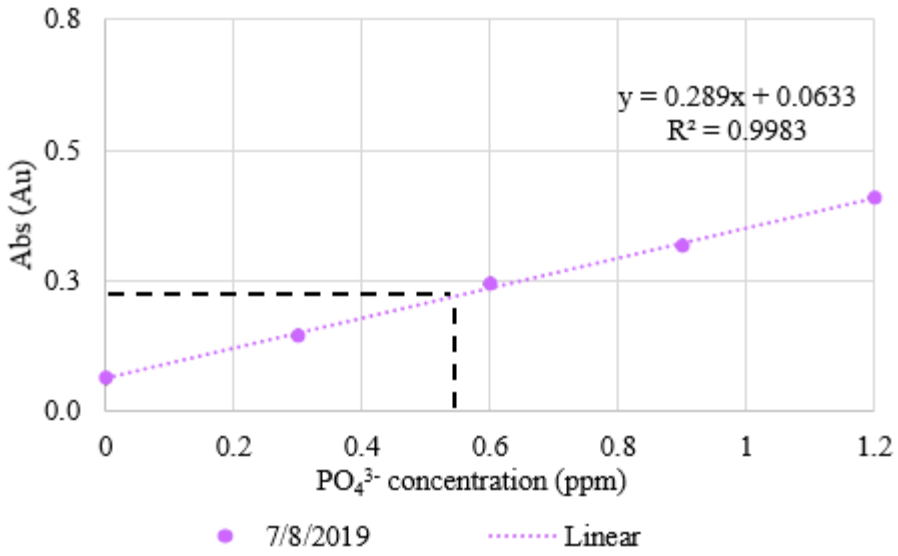

Figure 1.8 Calibration curve of phosphate standards using spectrometry. Linear fit is acquired for high reproducibility of method.

1.3.3. Turbidity

This analytical technique uses nephelometry, or the measurement of lost light intensity by scattering from particulates at a 90° angle. The premise is similar to a spectrophotometer except that rather than measuring absorbed light, the detectors quantify the light scattered at differing angles. As light is focused onto the sample light is scattered isotropically, while the remainder of light is directed to the main detector.[45] A transmitted light detector is located in line with the sample that measures the extent of light transmitted through the sample. Any attenuation of the beam is due to a combination of light scattering effect and absorption. A main detector is placed at a 90° angle from the light source to minimize transmitted light from the source reaching the detector. Forward and back scatter detectors are placed between the main detector and the transmitted light detector or the light source, respectively. These detectors are

present for high turbidity samples where a small amount of light is reflected in the direction of the incident light source.[45]

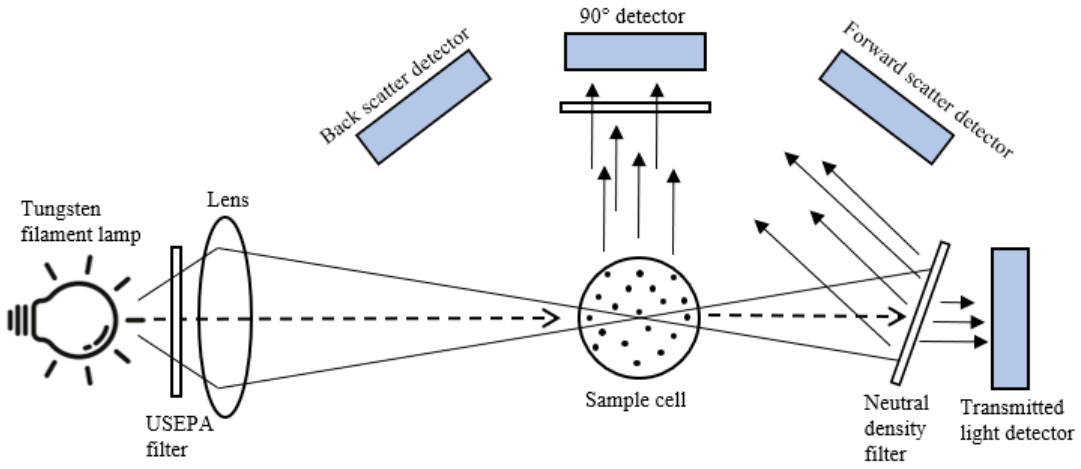

Figure 1.9 Schematic of 2100AN Turbidimeter that was used for turbidity analysis in this project.

Quantitation using turbidimetry utilizes the linear relationship between light scatter and sample turbidity, measured on a scale from 0 to 4,000 nephelometric turbidity unit (NTU).[46] This linear range is specific to the design of the instrument based on number of detectors and level of measurements. The turbidimeter used in this project has a ratiometric design. This design allows for multiple detectors that can minimize noise and color effects on NTU value. The unit of measurement for turbidity values is NTU which represents the clarity of water with suspended particle presence. Samples with a high NTU values are visually cloudy while samples with a low NTU value are visually clear.

1.3.4. Statistical Analysis

Two statistical techniques were used to analyze the dataset that included primary component analysis (PCA) and hierarchical clustering analysis (HCA). These

techniques take large datasets with variables and determines observations that correlate with each other. PCA was used as an exploratory data analysis tool to reduce the dataset into multidimensional space to define trends and patterns of the variables more clearly.[47] PCA extrapolates information to determine the amount of variance between the datapoints and how much the variables contribute to this variance. This information is assessed in multiple dimensions to define trends among the dataset. The results are displayed using a variety of visualization plots.

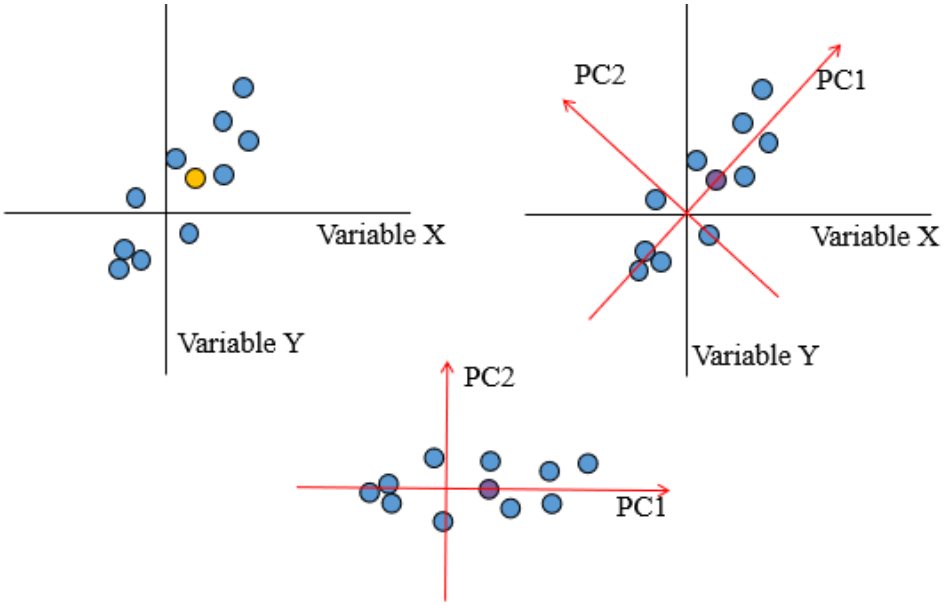

Figure 1.10 Example of PCA directional lines drawn for principal components.

This data is plotted on dimensional vectors to display linear combinations between the variables with maximum variance.[47] A line is displayed when PCA has determined the most variance within that direction of data points. These combinations are referred to as the principal components (PCs). The best fit line is drawn, containing the most information about variance within the dataset for a set of variables, in a dimensional direction which is defined as PC1. The second-best fit line is drawn

through the second highest telling linear combination. This line is drawn at a 90° angle of the first principal component (Figure 1.10.) The variance of the datapoints along these drawn lines is evaluated to display a percentage of information found within that component of the overall dataset.

Data points that aggregate on these drawn lines are considered to have a smaller variance, while more spread-out points have a larger variance. These results are displayed using graphical visualization techniques including monoplots and biplots. These techniques allow for easier data interpretation and visualization of the dataset on the whole. Other visualization graphs can be used for data interpretation but for the purposes of this specific dataset a monoplot and biplot were most appropriate.

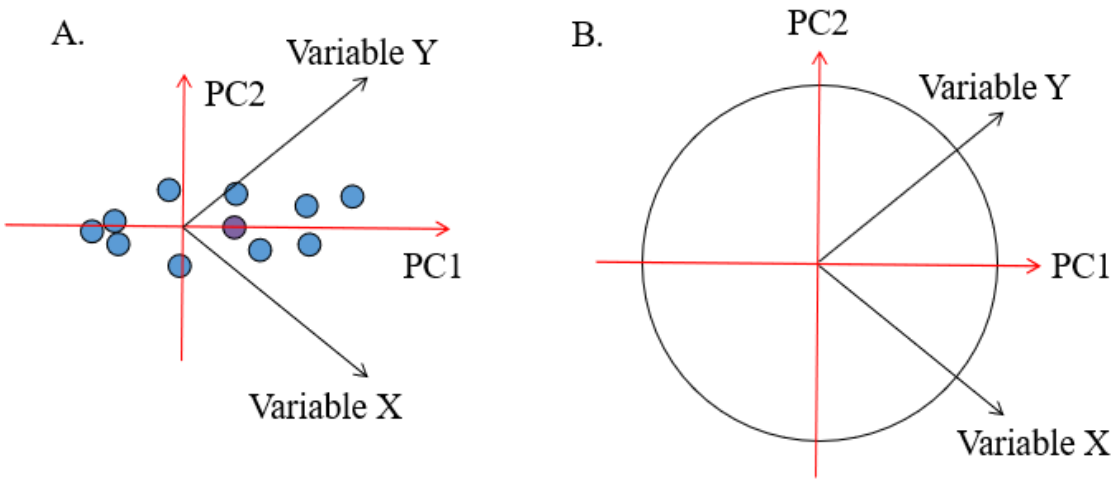

Figure 1.11 Example of PCA analysis A) biplot of individual observations first two principal components and B) monoplot of variables to show direction of loading.

Biplots display variable relationships and trends amongst datapoints on a single plot (Figure 1.11 A). The plot displays information from the most telling PCs while retaining multidimensional space. Using these visualization plots, similar data is expected to group together which would be displayed as clustered variable arrows on

the monoplot, or conglomerates of datapoints on a biplot. Biplots allow the user to establish clusters of similar characteristics by variable such as time collected or location. Monoplots display the relationships between variables in relation to one another within the dataset. The variable markers are represented as arrows that cluster based on similarities (Figure 1.11 B). Variables that are positively correlative are grouped together while those oriented orthogonal to one another are uncorrelated. Variables with negative correlation are plotted on opposing quadrant. The angle between the vectors represent the strength of correlation between the variables. For the purpose of this project, data points were chosen to be clustered by location expecting that locations would cluster together rather than spread out. The biplot displays a percentage value on the x and y axis that represents the amount of variance of the total.

Hierarchical Clustering Analysis (HCA) is used as an exploratory tool for data analysis to cluster data based on similarities by a ranking system of importance. The R program searches the dataset for underlying observations and clusters them into subsets to structure the dataset based on similarities. The ward linkage method is used in HCA which resamples the data by multiscale bootstrap resampling.

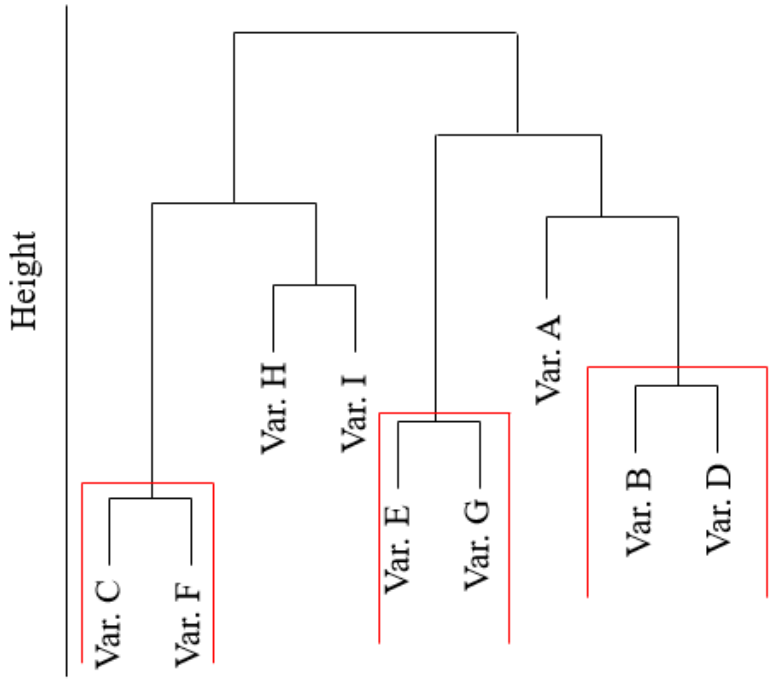

Figure 1.12 Example of HCA dendrogram of variables. Red boxes represent highly correlated variables associated with percentage values of AU/BP of each variable.

This method results in p values that are associated with calculations of hypotheses from the resampling of data.[48] This method is applied to HCA to allow the user to determine the purpose of clustering groups. Clustering is represented as a graphical dendrogram that visualizes the clustering of variables through a binary tree related to the dataset.[49] The dendrogram is formed by joining clusters together within their linkage to one another established by a vertical line. Those corresponding together is represented as connected by horizontal lines near the bottom of the binary tree. Established p values are calculated that indicate the strength of a cluster that's supported by the dataset. Red boxes are placed around clusters highly supported by the dataset.

1.3.5. Advantage of using multiple techniques

As this research focuses on harmful algal bloom formations in local water sources that haven't been previously monitored, it is important to validate any findings. Using multiple analytical techniques can allow results to be compared and confirm the quantitative values. Additionally, each piece of instrument has differing levels of sensitivity and selectivity that can be complementarily leveraged. The following chapter introduces this research's approach to experimental design which includes comparing EPA-validated analytical techniques with semi-quantitative testing kits that utilize the same instrumentation to determine authenticity of these semi-quantitative methods for lay persons.

Experimental Design

2.1. Sample Acquisition

Sample collection was conducted for two summers and involved monitoring environmental conditions at the sampling site followed by quantification of anion concentrations and turbidimetry in the lab.

2.1.1. Location Selection, Summer 2018

At the beginning of this project in Summer 2018, the aims of the initial sampling and water source monitoring were to examine varying types of water sources for algal bloom formation and observe any changes in the drinking water produced from these sources during an algal bloom, if available. As such, the locations selected included lakes (Lake Herrington and Lake Reba), a river (Kentucky River), and a small private pond (Doug Jackson's farm pond). Locations Lake Herrington and Kentucky River are drinking water sources where samples could be collected before the water treatment process and after. Within such selections, an array of variables were monitored to examine differences in water ecosystems. The results from Summer 2018 led to extensive changes in experimental design, framework for the project, and selection of locations leading into Summer 2019.

2.1.2. Location Selection, Summer 2019

In designing the approach for sample collection of Summer 2019, locations were narrowed down by water source type to focus primarily on private, manmade reservoirs, but the total number of locations was increased. Locations were chosen to be in close proximity to each other as those could be reasonably expected to have similar values for environmental variables (Figure 2.1). These locations includes sites in both Richmond,

KY and neighboring Waco, KY to the east. For the private pond reservoirs within Waco locations, approval from landowners was obtained using a consent form before sampling commenced. In addition to obtaining consent, private property owners were contacted to establish normal agribusiness conditions using a brief survey. It was emphasized to owners to continue agriculture practices as normal, but to contact the researchers if any fertilizers were to be applied to their property to accurately evaluate if any influence of added fertilizer. No property owners indicated actively using fertilizers on the property during sampling collection. Locations DJ1, DJ2, RD, and TJ contained livestock presence on the property, while a single property location (Doug Jackson Pond) contained previous biannual fertilizer use and livestock presence. The Doug Jackson Pond location contained three small farm pond reservoirs annotated as DJ1, DJ2, and DJ3. Livestock was moved annually between two pond areas (DJ1 and DJ2). This year livestock was present on location DJ1's property.

Figure 13 Satellite image of sampling locations utilized in Summer 2019. Water sources are classified based on type, influences, and presence of blooms. The inserts show enlarged areas with multiple closely-spaces locations.

Richmond locations were public water sources, so no approval was necessary to enter the location. Richmond locations included publicly accessible water sources with regular human and animal activity. The Lake Reba location was selected to be reevaluated from the previous summer to continue the investigation of influence of agricultural runoff from fertilizer use of surrounding farms and golf courses. The Stratton Pond location was expected to have some influence from human and animal activity, but not from fertilizer use or agricultural runoff as the property is managed by Eastern Kentucky University. This water source is categorized as recreational as students are encouraged to visit the pond filled with aquatic wildlife and take in the scenic view.

2.1.3. Sampling Techniques and Timelines

Sampling during 2018 occurred biweekly from late May through August. Samples were collected from each location at approximately the same time each day, starting at 8:00 am until collection was complete (approximately 4:00 PM). Samples were collected and stored using high density polyurethane (HDPE) containers in accordance with federal regulations for water sampling.[50] Samples were collected before, during, and after algal blooms to ultimately establish normal conditions from bloom events. Normal conditions were determined as nonalgal bloom days where visual observations of a bloom did not occur. Samples were collected at multiple depths including at the surface, 2 feet below, and 4 feet below the surface. Depth collection was applicable at all locations except for Doug Jackson Pond which was less than 2 feet deep. Sampling below the surface was achieved through the use of a Van Dorn water sampler which consists of a clear plastic cylinder connected to a suction device and

weight. This sampler allows a measured precision of the depth below the surface and the ability to remove a sample from that depth with minimal disruption to the water composition. For each acquired water sample, 250 mL of the sample was preserved using 2 mL of 18.4 M sulfuric acid and placed in an ice cooler during transport back to the lab where additional testing methods were conducted.

Sampling during 2019 occurred from June to late August. Samples were collected from each location at approximately the same time each day, starting at 7:00 am until collection was complete (approximately 10:00 AM). The close proximity of locations greatly reduced the sampling time of collection from the previous summer. Samples were collected each Monday, Wednesday, and Friday during the collection period. Normal conditions were determined as nonalgal bloom days where expected trends and visual observations of blooms didn't occur. Collected samples were obtained only at surface depth. Samples were otherwise collected and preserved according to the same procedure as in the Summer 2018 protocol.

2.1.4. Storage Conditions

Collected samples were stored based on federal regulation[50] which states preservation requirements based on method specificity and sample type. Sample preservation included being cooled to 4°C either with or without the addition of concentrated sulfuric acid. Preservation of samples ensures minimal chemical or biological changes to collected samples after removal from parent water source. The addition of sulfuric acid preserves the fully protonated form of phosphate (H_3PO_4) so the sample could be analyzed for additional forms of hydrolysable and total phosphorous concentration through digestion procedures.[50] Preservation with acid is not

necessary for samples analyzed by ion chromatography methods, only refrigeration.[50] Analytical testing for turbidity must be completed within 48 hours of collection, whereas samples evaluated by ion chromatography can be extended until 28 days within collection for most analytes including bromide, chloride, fluoride, and sulfate. Additional analytes which are required to be analyzed within 48 hours of collection include nitrate, nitrite, and orthophosphate.

2.2 Data Acquired at Point of Sampling

A set of environmental conditions and water quality aspects were monitored at each location during sampling collection. This included variables such as pH, conductivity, air temperature, water temperature, dissolved oxygen, and turbidity.[51] Additional conditions such as visual observations of water and weather patterns, stream flow, stream mixing and notes of activity by human or aquatic life were conducted at each location as described in previous literature.[2, 52]

2.2.1. Measurement of Dissolved Oxygen

Dissolved oxygen refers to the amount of oxygen gas available in water. Oxygen in water is generated by flow or movement in water or as a byproduct of photosynthesis. This variable has been investigated as a potential indicator for harmful algal bloom formation.[53-55] Sustainable conditions for thriving aquatic life require dissolved oxygen levels at or above 6 mg/L.[53] Measurements above this value support healthy growth and activity conditions for aquatic life, while below this level indicates an anoxic environment.[53] During an algal bloom event, a decrease in dissolved oxygen is expected from normal conditions.[9] There is no known approximate amount of decrease expected for dissolved oxygen during an algal bloom event as different water sources experience

bloom events near hypoxia (2 mg/L)[53] or anoxic (0.5 mg/L) conditions[53], while other can contain values near 5 mg/L to 6 mg/L. In addition to algal bloom effects, an annual trend is predicted with a decrease in dissolved oxygen as air and water temperature increase during summer months.[9] The relationship between dissolved oxygen and temperature is indirect, so as temperature increases, the solubility of dissolved oxygen decreases. Higher temperature water requires less available dissolved oxygen necessary to reach saturatation.[54] Dissolved oxygen was measured using a LaMotte Dissolved Oxygen Test Kit (LaMotte Company, Chestertown and Maryland). This titrimetric method involves multiple steps and additions of reagents to determine the saturation of oxygen present (Figure 2.2). The reactions involved are summarized in equations 2.1-2.5.

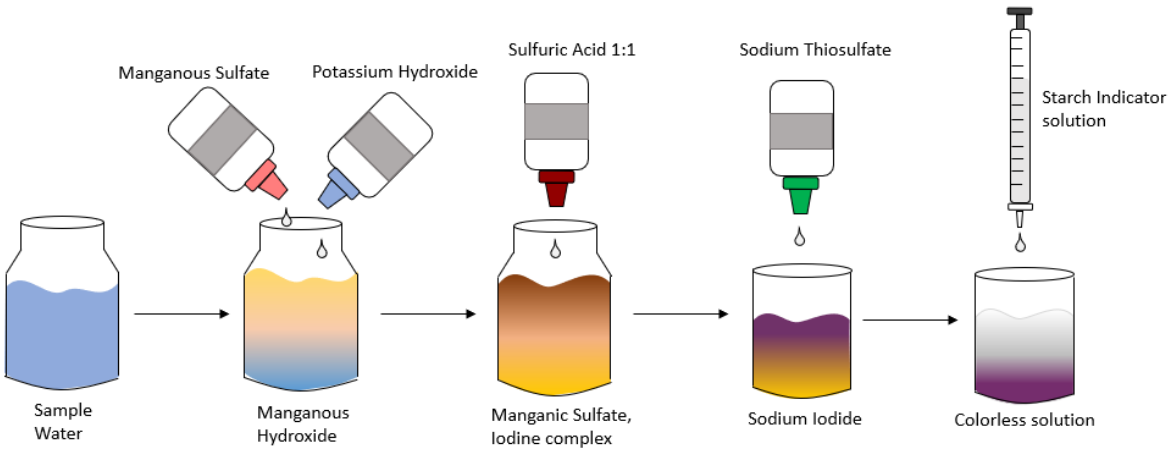

Figure 14 Overview of procedure and expected color changes during the measurement of dissolved oxygen using a LaMotte water quality test kit.

$$MnSO_4(aq) + 2KOH(aq) \rightarrow Mn(OH)_2(s) + K_2SO_4(aq) \qquad \text{Equation 2.1}$$

$$4Mn(OH)_2(s) + O_2(g) + 2H_2O(aq) \rightarrow 4Mn(OH)_3(s) \qquad \text{Equation 2.2}$$

$$2Mn(OH)_3(s) + 3H_2SO_4(aq) \rightarrow Mn_2(SO_4)_3(aq) + 6H_2O(aq) \qquad \text{Equation 2.3}$$

$$Mn_2(SO_4)_3(aq) + 2KI(aq) \rightarrow 2MnSO_4(aq) + K_2SO_4(aq) + I_2(aq)$$ Equation 2.4

$$2Na_2S_2O_3(aq) + I_2(aq) \rightarrow Na_2S_4O_6(aq) + 2NaI(aq)$$ Equation 2.5

Manganous sulfate and potassium hydroxide react to form a pale, yellow colored precipitate of manganous hydroxide (Equation 2.1).[55] After an oxidation process occurs, manganic hydroxide forms in a brown precipitate that floats in the solution (Equation 2.2).[55] Free iodine is converted to sodium iodide with the addition of sodium thiosulfate resulting in the solution turning dark purple (Equation 2.5).[55] The limiting reagent is introduced to measure the available depleted oxygen in the water source. A standard starch solution is added drop-wise to the solution until clear. This titrimetric reaction involves the visualizing the endpoint so accurate measurements can be made. Dissolved oxygen measurements can be read with this kit from 0 to 14 mg/L. Premade solutions and measurements directed from LaMotte procedures were used for this testing kit measurement.

2.2.3 Measurement of pH

The pH of a solution is a measurement of the amount of available free hydrogen and hydroxide ions present in the water source. Acidic water contains more hydrogen ions, basic water contains more hydroxide ions. As algae decays, hydroxide ions are released into the water.[56] The pH of each water source remained in the expected range of 6-9 for sustainable aquatic life. Monitoring this variable was achieved through a colorimetric, semi-quantitative method. A HACH pH test kit (HACH, Loveland, Colorado) which utilized a pH indicator solution in combination with a color comparator disk was used to determine the pH of each water sample as shown in Figure 2.3. Following the kit procedure, each water sample was given 6 drops of pH indicator

solution placed in the sample cell on the left. The color comparator disk is compared to the reference sample in the right sample cell containing only sample water. While less precise than a pH meter, this kit was robust and allowed pH measurements to be obtained immediately after sample collection.

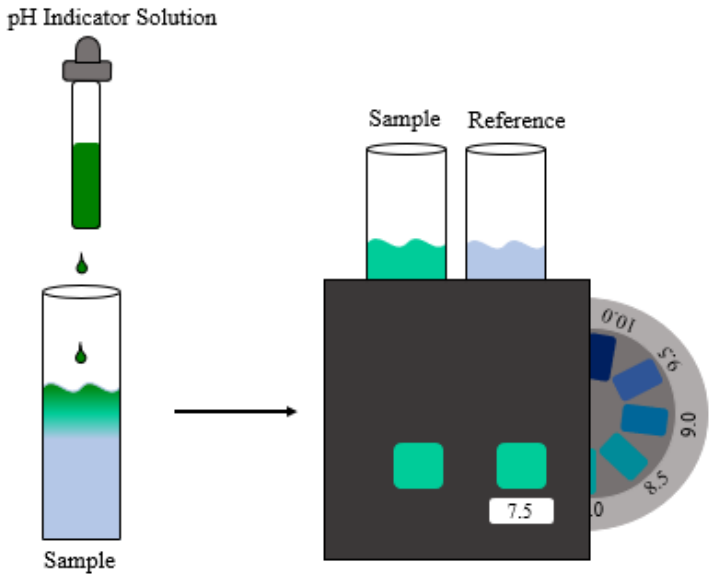

Figure 15 Procedural diagram of HACH pH reaction

2.2.4 Measurement of Conductivity

Conductivity measures the ability of flow of current from via ions in the water source and can be used to quantify, which ultimately indicates the relative amount of salts and inorganic material. Monitoring changes in conductivity can indicate a shift or disturbance in the environment. An Oakton EcoTestr EC pocket conductivity meter (Cole-Palmer, Vernon Hills/Illinois) was used to measure conductivity in the range of 0-1990 micro Siemens (µS). An increase in conductivity is expected to be observed as temperature increases where conductivity of 2-4% in measured values will increase for every 1°C increase.[57]

2.2.5 Measurement of Temperature

Air and water temperature were monitored by digital and handheld thermometers, respectively at each location and each time point. It was expected that as air temperature increases, so does water temperature. More heat is required for raising water temperature than air temperature due to the differing heat capacities.

2.2.6 Visual Turbidity

Turbidity at the time of collection was visually assessed through field observations based on Kentucky Watershed Watch guidelines.[58] In water quality field observations, turbidity is based on visual point scale where 0 represents clear water with little particulate presence and 3 represents poor clarity with large amounts of particulate presence.

2.2.7 Rainfall

Rainfall was monitored in the vicinity of each location through a local Kentucky Mesonet rain gauge station known as Richmond 8 E (ELST).[58] This station is located approximately 8.9 miles from Richmond and 4.6 miles from Waco. Values were recorded in inches of rain for each 48-hour period.

2.3. Taxonomic Identification

Performed on samples suspected to contain an algal bloom once returning to the laboratory was taxonomic identification. This was used to identify species of algae and other microorganisms present during an algal bloom through their morphology. Taxonomic identification was performed on samples from days that visually appeared to have blooms. Samples were mounted on glass slides with coverslips and analyzed using a compound light microscope (Premiere, C&A Scientific) which uses a light

source directed through a series of lenses to magnify visual components of the sample. Morphological identifications were determined by using reference samples and additional reference guides[60-62] that provided microscopic images of various algal species.

2.4. Quantitative/Analytical Methods

A series of analytical techniques were used to quantify nutrient concentration in samples including spectrophotometry, ion chromatography, and turbidimetry. Methods for analysis of phosphorous, nitrogen, and various ion species from approved EPA documentation.[63] Method validation prior to and during sample analysis included using external standards and calibration curves. In addition to the method described in the remainder of this section, a method for measuring total Kjeldahl nitrogen did not meet validation requirements and was not implemented in this project.

2.4.1 Quantitation of Phosphate Using Spectrophotometry

The method used for analysis of phosphorous was EPA method 365.3 otherwise known as "Phosphorous, All Forms (colorimetric, ascorbic acid, two reagent)".[64] This method involves the measurement of orthophosphate, hydrolyzable phosphorous, and total phosphorous using 3 separate digestion procedures, briefly described in Figure 2.4. In each case, the phosphorous present is converted into a blue-colored antimony-phospho-molybdate complex. The intensity of the colored solution is proportional to the concentration of phosphorous in the solution. Other forms of phosphate can be converted to orthophosphate through additional reactions: hydrolyzable phosphorous is

broken down by sulfuric acid hydrolysis, while total phosphorous is broken down by a persulfate digestion procedure.

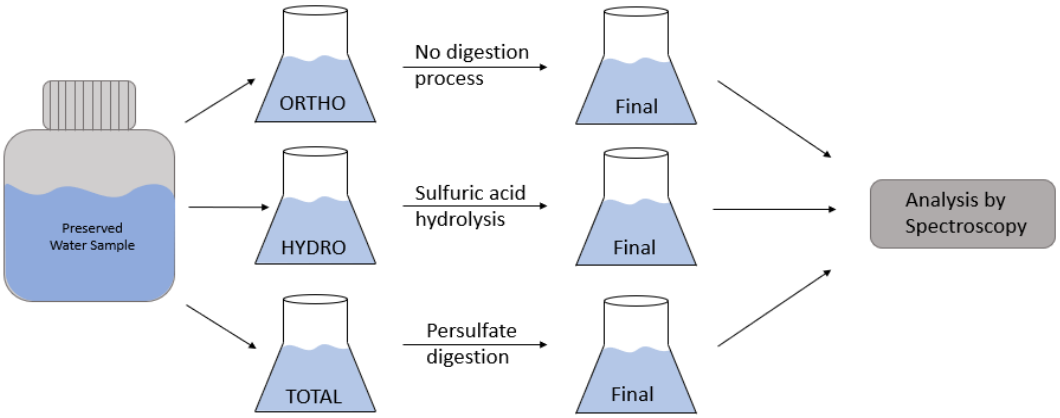

Figure 16 Procedural diagram of phosphates analysis, method 365.3.

Validation of this method consisted of performing multiple trials using calibration standards in order to confirm the method's reproducibility. The EPA method indicated a dynamic range of 0.1 to 1.2 mg/L of phosphate.[63] Calibration standards were prepared using a range of phosphate concentrations extending from the lower limit through the higher limit of quantitation of the method. Extending the range of concentration allowed for minimum detection limits to be determined spectroscopically. Within this method, parameters were set to determine phosphorous concentration at 650 nm. Replicate measurements were performed to assess reproducibility of mixtures from the three separate test methods. An R^2 value greater than or equal to 0.98 was used to ensure linearity throughout the calibration range (Figure 2.5). Equation of lines should be similar across daily testing to convey reproducible results. This includes a highly linear relationship equating a high R^2 value. Matrix interference checks were performed by using a spiked sample to determine any inhibition occurrence in collected water.

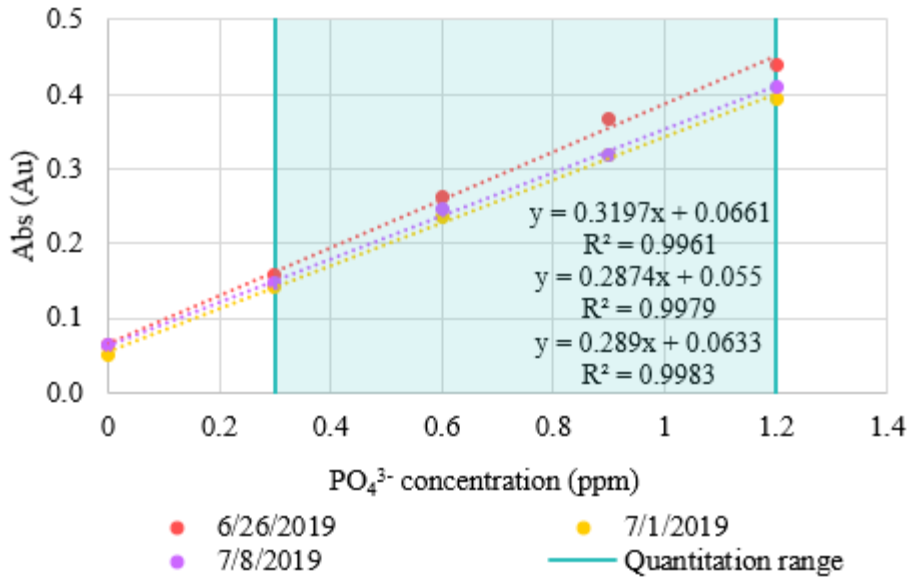

Figure 17 Calibration curves of 3 separate days for phosphorous analysis using EPA method 365.3. The teal region represents the quantitation range for this method.

2.4.2 Quantitation of Inorganic Anions Using Ion Chromatography (IC)

IC was used to quantify the concentration of the following anions: fluoride, chloride, nitrite, bromide, sulfate, nitrate, and orthophosphate. This technique was based on EPA Method 300.0. Standard calibration solutions were analyzed with every batch of samples to enable data for quantitation. These solutions contained varying amounts of each analytes, as shown in Table 2.1. The amount of each anion was selected based on the recommended concentration range for each analyte and optimized so as not to exceed the maximum anion concentration allowed by the column. For each analyte a calibration curve was generated using the peak area of each analyte peak. Calibration ranges were extended beyond higher and lower limits of the EPA method to determine LOD of the method while preserving an R^2 value of ≥ 0.98. Examples of the calibration

curves generated for the sulfate anion over several dates across the time frame of this study are shown in Figure 2.6.

Table 2.1 Composition of calibration mix standards utilized for anion quantitation using ion chromatography.

	Fluoride	Chloride	Nitrite	Bromide	Sulfate	Nitrate	Orthophosphate
Mix G	0.20	0.60	0.20	25.00	100.00	0.30	0.50
Mix H	0.80	3.00	1.00	10.00	60.00	1.00	2.00
Mix I	2.00	9.00	5.00	5.00	35.00	6.00	5.00
Mix J	6.00	15.00	10.00	2.00	10.00	13.00	10.00
Mix K	10.00	30.00	15.00	0.50	2.00	20.00	25.00

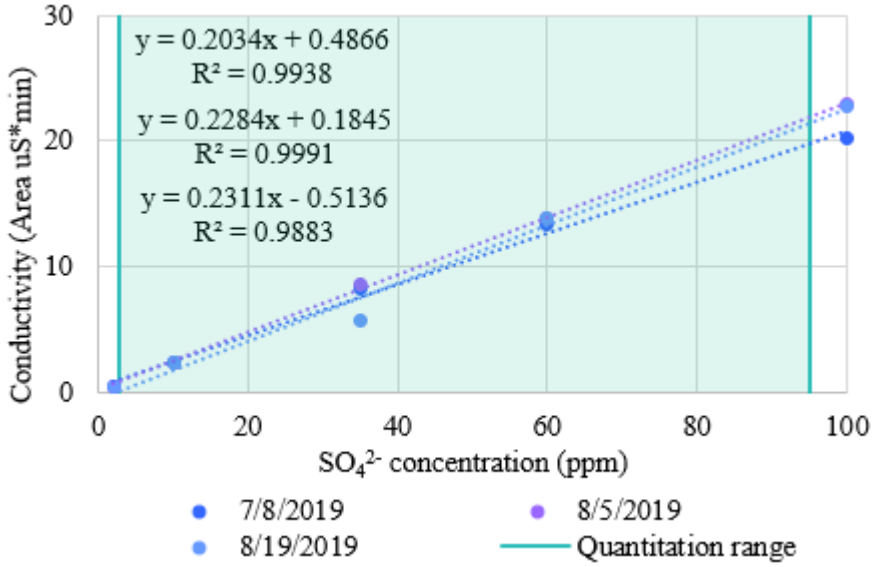

Figure 18 Calibration curves generated for ion chromatography on three separate days analyzing sulfate levels. The teal region represents the quantitation range specified by EPA Method 300.0.

2.4.3 Turbidity

Turbidity was used to quantify the intensity of scattered light in a sample that contains suspended solids. EPA method 180.1, Determination of Turbidity by

Nephelometry,[65] was applied to water samples to quantify clarity and define readings by nephelometric turbidity units (NTU) using a 2100AN Turbidimeter (HACH, Loveland, Colorado). Per this method, the instrumentation is required to be calibrated for turbidity range 0 to 4000 NTU every 90 days with use of a primary standard suspension of formazin (Sigma-Aldrich, Milwaukee, Wisconsin). A secondary standard suspension mixture of formazin and distilled water was used as a daily calibration check. An additional blank sample containing only distilled water was used during each run to ensure no contamination was present. Samples were analyzed for turbidity within 48 hours of collection and the turbidity measurement associated with the clarity of each water sample recorded in NTUs. No further data treatment was necessary.

Visual turbidity in the field included assessing water quality based on clarity of water. Although visual turbidity assessment was double checked in the lab for quantitated data, there is a visual scale that correlates to analytical values received in the quantitated data based on this projects results (Table 2.2). This scaling model was used to assess approximate values associated with visual assessment and verbal description to lab results.

Table 2.2 Composition of visual cues utilized for assessment of visual turbidity correlating to analytical evaluation using turbidimetry.

Visual Assessment (0-3)	Verbal Description	Approximate Analytical values (NTU)
0	Crystal Clear	Values 0 to 10
1	Clear	Values 11 to 50
2	Cloudy	Values 51 to 80
3	Muddy	Values above 80

2.5. Method Validation

2.5.1 Ion Chromatography

Method validation involved assessing reproducibility of this method through multiple trials of standards. Each analyte of interest was used with different ranges in concentration as suggested by the method guidelines. Linearity of the resulting calibration curves for standards were evaluated and expected to be greater than or equal to 0.98. Matrix interference checks were performed using an internal standard design to assess possible matrix inhibition of analyte concentrations. There were no interferences from matrix inhibition of the water samples.

Table 2.3 Analytical parameters for the regression equations of the EPA ion chromatography method.

Parameter	Fluoride	Chloride	Nitrite	Bromide	Sulfate	Nitrate	Orthophosphate
Detection range (ppm)	0.0017-10.00	0.0303-30.00	0.0606-15.00	0.5-25.00	0.0371-54890.00	0.0406-20.00	0.1571-25.00
Linearity range (ppm)	0.20-10.00	0.6-30.00	0.2-15.00	0.5-25.00	2-100.00	0.3-20.00	0.5-25.00
Regression equation:							
Correlation coefficient (r)	0.9999	0.9992	0.9968	0.9954	0.9918	0.9998	0.9994
Intercept (b)	-0.4703	-0.8857	-0.2692	1.0174	2.9741	-0.0649	-0.0521
Slope (m)	2.3941	3.6144	1.8982	9.7232	16.6180	1.4345	3.8849
LOD (ppm)	0.0017	0.0303	0.0606	0.50	0.0371	0.0406	0.1571
LOQ (ppm)	0.20	0.60	0.20	0.50	2.00	0.30	0.50

2.5.2 Spectroscopy

Similar to the ion chromatography method, method validation involved assessing reproducibility of this method through multiple trials of standards within each testing method. The standard calibration range was evaluated using the breakdown procedure for all phosphate forms covered in this method. Linearity of standards were evaluated and expected to be greater than or equal to 0.98. Matrix interference checks

were performed using standard addition. There were no interferences from specifically matrix inhibition of the water samples.

Table 2.4 Analytical parameters for the regression equations of the EPA spectroscopy method.

Parameter	Hydrolyzable	Orthophosphate	Total Phosphate
Detection Wavelength (nm)	650	650	650
Linearity range (ppm)	0-1.2	0-1.2	0-1.2
Regression equation:			
Correlation coefficient (r)	0.9869	0.9989	0.9812
Intercept (b)	0.0547	0.0501	0.0232
Slope (m)	0.308	0.3128	0.568
LOD (ppm)	0.006	0.008	0.02
LOQ (ppm)	0	0	0

2.6. Semi-Quantitative Methods

A set of semi-quantitative techniques were used alongside the quantitative methods to validate commercially available test kits for use by the lay person. In general, semi-quantitative test methods require measurements by visual observation which are compared with a standard chart of expected results for each range of analyte concentrations. The visual observations may be assessing the color or turbidity of the sample. In order to ensure reproducibility of the methods and to attempt full quantitation using the kits, each method was validated through available instrumentation using standard calibration curves. Acquired data from semi-quantitative measurements were compared with quantitative test results to confirm accuracy. Testing kits included kits for the analysis of phosphates, nitrates, nitrites, and sulfates. In addition to the nutrient kits, a semi-quantitative *E. coli* test was also performed. Due to limited reagents for each test, select dates and locations were used as a comparison study between semi-quantitative test kits and analytical techniques as well as for *E. coli*.

2.6.1 Phosphates Semi-Quantitative Kit

A colorimetric-based test kit (Taylor Technologies, Sparks, Maryland) was used for semi-quantitative phosphate measurement. This semi-quantitative method utilizes a color card comparator. To attempt quantitation using this kit, spectrophotometric absorbance measurements were obtained at wavelengths corresponding to the color of the final solution. Calibration standards in a concentration range of 1 to 6 ppm were used to determine the reproducibility of testing methods. The test kit uses phosphate reagents including a mixture of sulfuric acid and sodium molybdate (reagent #1), glycerol (reagent #2), and sodium thiosulfate which react to form orthophosphate complex that is proportional to concentration of the analyte (Equations 2.6). Molybdophosphoric acid is converted to a phosphomolybdate complex which is reduced by the amino acid method which can be seen in equation 2.6.[66]

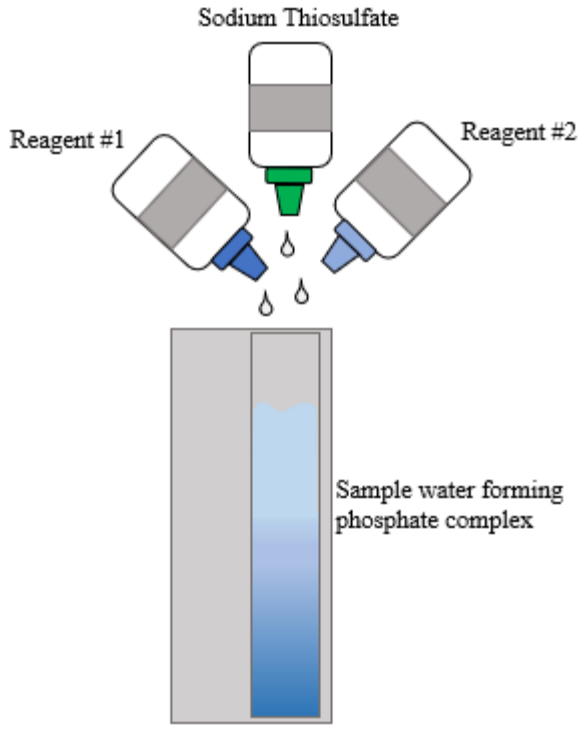

Figure 19 Overview of procedure and expected color changes during the measurement of orthophosphate using Taylor Technologies testing kit.

The absorbance of the resulting complex was analyzed using UV-VIS spectroscopy at 650 nm. The calibration range for the semi-quantitative kit was 0 to 1 ppm, similar to that of the quantitative method. Samples were unfiltered to replicate what would be available in the field when conducting tests.

$$12MoO_3(aq) + H_2PO_4^-(aq) \rightarrow (H_2PMo_{12}O_{40})^-(aq)$$ Equation 2.6

2.6.2 Nitrates Semi-Quantitative Kit

A colorimetric-based test kit (HACH, Loveland, Colorado) was used for semi-quantitative nitrate measurement. Nitrate concentration was quantified using semi-quantitative measurements by color card comparator and quantitatively using spectrophotometric measurements set at 500 nm. Calibration standards in a concentration range of 0 to 40 ppm were used to determine analyte concentration in

unknown samples. Calibration standards were extended to 200 ppm to test validity outside of recommended range of testing kit. Samples were unfiltered to replicate what would be available to lay persons in the field when conducting tests.

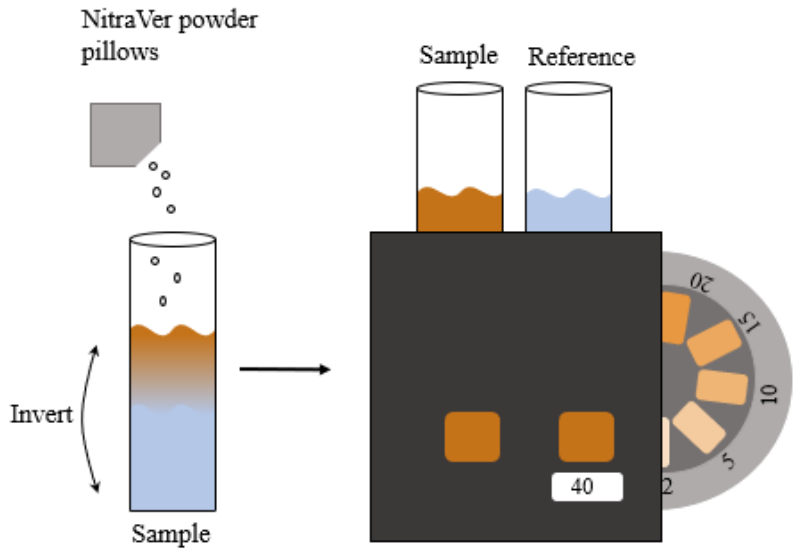

Figure 20 Overview of procedure and expected color changes during the measurement of nitrate using HACH testing kit.

The test kit used nitrate reagents to react cadmium particles from the pillow pocket to form a nitrate complex in the sample that is proportional to concentration of the analyte. This process is described in equations 2.8 and 2.9.[67]

$$NO_3^-(aq) + Cd(s) + 2H^+(aq) \rightarrow NO_2^-(aq) + Cd^{2+}(s) + H_2O(aq) \qquad \text{Equation 2.8}$$

$$NO_2^-(aq) + H_2N - C_6H_7NO_3S - SO_3H(aq) + 2H^+(aq) \rightarrow HO_3S - C_6H_5N_2^+ - N(aq) + 2H_2O(aq) \qquad \text{Equation 2.9}$$

2.6.3 Nitrites Semi-Quantitative Kit

A colorimetric-based test kit (HACH, Loveland, Colorado) was used for semi-quantitative nitrite measurement. Similarly, to phosphate and nitrate, nitrite concentration was quantified using semi-quantitative measurements by color card

comparator and quantitatively using spectrophotometric measurements at 550 nm. Calibration standards in the range of 0 to 80 ppm were used in determining unknown analyte concentration in water samples. Calibration standards were extended to 200 ppm to test validity outside of recommended range of testing kit. Samples were unfiltered to replicate what would be available to lay persons in the field when conducting tests.

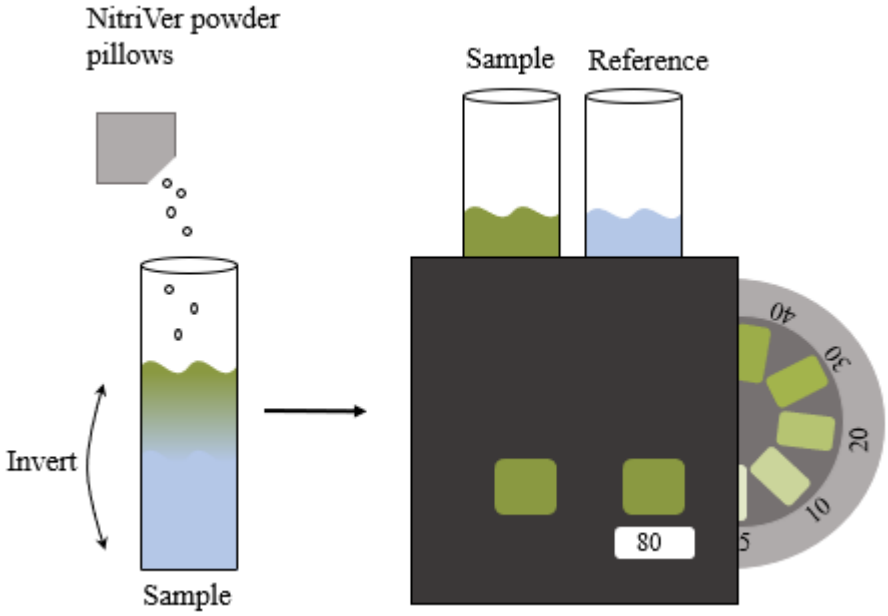

Figure 21 Overview of procedure and expected color changes during the measurement of nitrite using HACH testing kit.

The test kit used nitrite reagents to react ferrous sulfate particles from the pillow pocket to form a nitrite complex in the sample that is proportional to concentration of the analyte.[68] This testing kit uses a ferrous sulfate reaction which can be seen in equation 2.10 and 2.11.

$$2Fe^{2+}(s) + 4H^{+}(aq) + 2NO_2^{-}(aq) \rightarrow 2Fe^{3+}(s) + 2NO(aq) + 2H_2O(aq) \quad \text{Equation 2.10}$$

$$NO(aq) + FeSO_4(aq) \rightarrow FeSO_4 \cdot NO(aq) \quad \text{Equation 2.11}$$

2.6.4 Sulfates Semi-Quantitative Kit

A turbidimetric-based test kit (HACH, Loveland, Colorado) was used for semi-quantitative sulfate measurement. Sulfate concentration was quantified using semi-quantitative measurements by extinction dipstick comparator and quantitatively using turbidimetry measurements. Calibration standards in a range 0 to 200 ppm were used for determination of unknown concentrations of analyte in samples. Calibration standards were extended to 500 ppm to test linear response outside of recommended range of testing kit. Similar calibration ranges were used for both analytical techniques and semi quantitative test kits. Sulfate test kit samples were analyzed using turbidimetry. Samples were unfiltered to replicate what would be available to lay persons in the field when conducting tests.

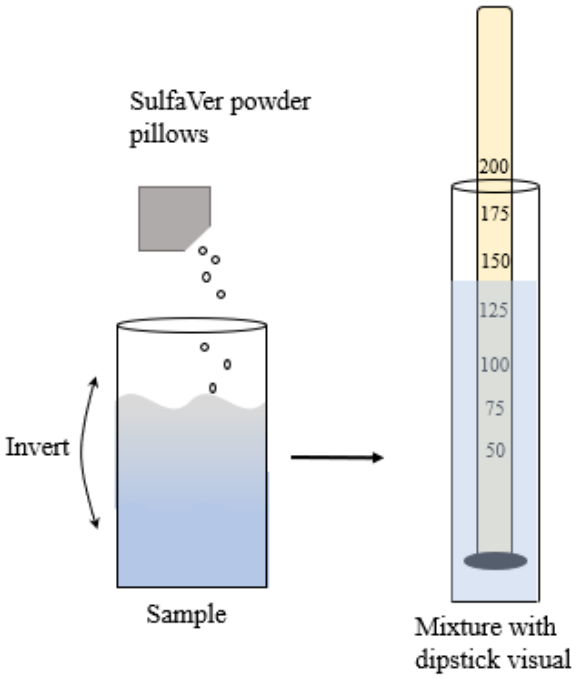

Figure 22 Overview of procedure and expected color changes during the measurement of sulfate using HACH testing kit.

Test kit uses sulfate reagents including a sulfate powder pillow which reacts with barium chloride in water to form a barium sulfate complex that is proportional to concentration of the analyte. This testing kit uses a barium sulfate reaction which can be seen in equation 2.12.[69]

$$Ba^{2+}(s) + SO_4^{2-}(aq) \rightarrow BaSO_4^{-}(aq) \qquad \text{Equation 2.12}$$

2.6.5 Escherichia coli (E. coli) Semi-Quantitative Kit

E. coli was monitored to rule out animal fecal matter as the primary source of fluctuations in nutrient levels leading to algal bloom formation. Semi-quantitative testing methods were used to determine most probable number (MPN) of *E. coli* colonies present in 100 mL of water sample after incubation. Colilert testing (IDEXX Laboratories, Inc., Westbrook, Maine) was used to detect and quantify total coliforms and Escherichia coli (*E. coli*) within 24 hours. This test detects nutrient indicators, ONPG (ortho-nitrophenyl-beta-D-galactopyranoside) and MUG (4-methylumbelliferyl-beta-D-glucuronide), that are sources of carbon which are later metabolized by the coliform enzyme β-galactosidase and *E. coli* enzyme β-glucuronidase.[70] As the bacteria grows, the β-glucuronidase enzymes metabolize the nutrient indicator ONPG which turns the solution color from colorless to yellow. *E. coli* uses β-glucuronidase to metabolize the other nutrient indicator, MUG, which results in fluorescence.[70] A Quanti-Tray system is used to quantify bacterial counts of 100 mL sample sectioned into 51 sample wells into a compressed mold. The IDEXX reagent is mixed with the sample where it is poured into a Quanti-tray mold. This mold is sealed by the Quanti-tray system and incubated for 24 hours at 35-37°C. The water sample molds are incubated for 24 hours then the vibrant yellow or fluorescent positive wells are counted.

Total coliform wells are considered positive by a vibrant, yellow color while E coli wells are positive by fluorescent wells under long-wave UV light (366 nm).[71] Using the number of positive wells, the most probable number (MPN)[71] model was used to quantitate number of bacteria in 100 mL of sample. The MPN model is used to estimate the concentration of microorganisms in a sample by replication of growth in ten-fold dilutions.[71]

2.7. Statistical Analysis

Data mining was completed using R statistical software (RStudio, Auckland, New Zealand) to interpret the collected dataset. The dataset used for PCA and HCA analysis included sampling locations and dates, weather patterns, measured variables in the field, and in lab quantified data. To prevent overloading the software program, the dataset was reduced to include every other sampling event data. These incorporated days affected by algal blooms and those not affected by them, to ensure enough data with blooms and without were evaluated to determine trends among variables and datapoints. Nine variables (air temperature in Fahrenheit, water temperature in Celsius, dissolved oxygen, pH, conductivity, rainfall, turbidity, visual turbidity, sulfate levels by IC) were compatible with PCA as continuous, numeric data was required for accurate results. Expectations of data mining were to clearly define correlative variables within this dataset. R statistical software used the PCA{FactoMineR} command and library package to perform PCA on this dataset. The dataset was normalized before analysis to ensure data is consistent in the same format and contain the same standard deviation. Data analyzed using PCA is required to be normalized for accurate weight distribution for visualization. PCA was used to establish correlations between the original variables

and associated PC. Specifically, a linkage clustering system was used to cluster nine variables of the dataset to establish similarities. HCA was performed using the hclust command to establish clusters using dissimilarity and linkage functions through R statistical software. HCA was performed on the same nine variables previously analyzed through PCA using this program. The dataset was normalized before HCA was performed using this program.

Results & Discussion

3.1. Summer 2018 Data

3.1.1 Environmental Conditions

Sampling frequency was low during this summer, though the expectation was that observations would define trends clearly. During sampling, there was one algal bloom event observed at location DJ with no algal blooms observed in other locations. Location DJ contains one less data point than other locations due to addition of the location after first sampling event. While seasonal variations were expected throughout the summer, some variables observed diurnal variation in measurements. This means such variables respond to fluctuations that occur during each day from natural processes. This variation affects temperature, dissolved oxygen, pH and conductivity.

Measurements of pH during summer 2018 indicated some fluctuation but no more than what was expected from natural processes of respiration and photosynthesis. No significant changes were observed for locations KR, LR, and LH as measurements varied by <1 throughout the summer. A significant change in pH is constituted as a pH change to outside of the normal range for water (6.5 to 8.5). Aquatic environments with values below or above this range could result in unsustainable conditions for wildlife.[55] The one location affected by an algal bloom observed an increase in pH at the time of the bloom and the next sampling event (Figure 3.1A). This was expected for a bloom event as the pH will increase due to the decay of algae. As the bloom formation dies, it removes carbon dioxide from the photosynthetic cycle. Hydroxides are released back into the water source creating a more basic environment which is seen as an increase in

pH.[71] Thus, pH can remain higher in the water source until that algal bloom is disrupted, often from natural weather patterns including rain.

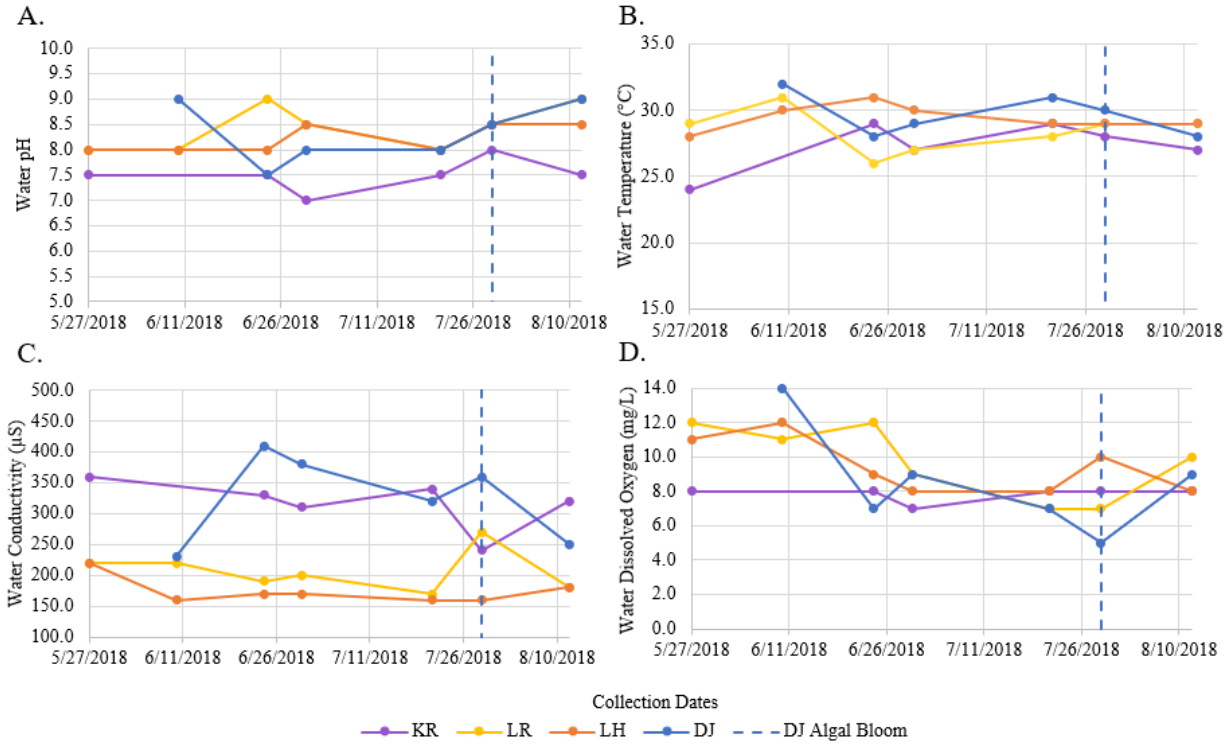

Figure 23 Environmental conditions for summer 2018 locations including A) pH, B) water temperature, C) conductivity, D) dissolved oxygen.

The water temperature of collected water samples showed no clear trend across all locations (Figure 3.1 B). Water temperature values were expected to increase through the traditionally warmer summer months. Temperature fluctuations were observed between sampling events. Diurnal variations were expected for this variable as measurements will vary depending on time of day.[72] To account for this water sources were sampled near the same time during each sampling events. There were significant changes in temperature that indicated disruption to the expected trend of warming temperatures that may be attributed to variable weather conditions. Locations with storm conditions on the date of sampling resulted in more fluctuation in air and water

temperature. Storm events occurred at location LH on 05/27/2018 and at locations DJ and LR on 06/10/2018 (Table 3.1). These events had the most distinguishable effect on location DJ. Fluctuation in the sampling times also appeared to affect the measured temperature, with locations sampled near the warmest part of the day containing higher temperatures than early morning or evening sampling sites. Location JP on 06/24/2018 was sampled at 09:50 am with a measured water temperature of 28°C. On the same day location LH was sampled at 01:10 pm with a measured water temperature of 31°C. Unfortunately, the spatial distribution of the sampling sites across 49.9 miles and access at some points requiring traveling by canoe made having a relatively uniform sampling time of day and weather conditions unattainable. These aspects were considered in the Summer 2019 sampling plan by narrowing the spread of locations studied to 19.9 miles. It was expected that temperatures above 25°C and increased light promotes growth of Cyanobacteria and leads to algal bloom formation.[25] Bloom location DJ observed optimal temperatures throughout the summer promoting growth of algae in the water source. This location contained agribusiness presence on the property whereas other sampled locations did not. This water source is relatively smaller and more private than other sample locations while containing agribusiness presence on the property as well. Other sample locations did not contain as prominent agricultural presence on properties. Locations LR, KR, and LH contained larger water sources with surrounding private and public properties that contained various influences from recreational and agricultural activity. These locations did not observe algal bloom formations in their water sources during the sampling collection events. Location DJ did experience an algal bloom formation during the sampling time frame. Agricultural runoff from agribusiness

activities such as livestock farming on Location DJ's property was initially hypothesized to contribute to algal bloom formation but could not be confirmed by the Summer 2018 data. However, this hypothesis helped frame the research objectives for following summer and the selection of locations to include those with similar variables as location DJ. The sampling frequency was also increased to more clearly define necessary conditions for bloom formations.

Table 3.1 Weather patterns for summer 2018 locations.

Sampling Site	Date	Water Color	Stream Mixing	Weather	Wind	Stream Flow
JP	6/10/18	Brown	Fair	Storm	Strong	Flowing Slow
JP	6/24/18	Brown	Fair	Clear, Hot	None	Stand Still
JP	6/30/18	Brown	Fair	Clear, Hot	Calm	Stand Still
JP	7/21/18	Green	Poor	Clear, Cool	None	Stand Still
JP	7/29/18	Brown/Green	Fair	Clear, Hot	Calm	Stand Still
JP	8/12/18	Brown	Fair	Clear, Hot	Calm	Stand Still
KR	5/27/18	Green	Good	Clear, Cool	None	Stand Still
KR	6/24/18	Green	Fair	Cloudy, Cool	Calm	Stand Still
KR	6/30/18	Brown/Green	Fair	Cloudy, Hot	Calm	Flowing Slow
KR	7/21/18	Brown/Green	Poor	Cloudy, Hot	None	Flowing Slow
KR	7/29/18	Green	Fair	Clear, Hot	None	Constant
KR	8/12/18	Brown/Green	Good	Clear, Hot	None	Flowing Slow
LR	5/27/18	Green	Good	Clear, Cool	None	Stand Still
LR	6/10/18	Clear	Fair	Rain, Storm	Breeze/Strong	Flowing Slow
LR	6/24/18	Green	Poor	Warm, Clear	Calm	Flowing Slow
LR	6/30/18	Green	Poor	Clear, Cool	Calm	Flowing Slow
LR	7/21/18	Green	Poor	Cloudy, Hot	Strong	Stand Still
LR	7/29/18	Brown/Green	Poor	Clear, Hot	None	Constant
LR	8/12/18	Green	Fair	Hot	Breeze	Constant
LH	5/27/18	Green	Fair	Storm	Calm	Flowing Slow
LH	6/10/18	Green	Good	Hot	Breeze	Constant
LH	6/24/18	Green	Fair	Clear, Hot,	Breeze	Flowing Slow
LH	6/30/18	Green	Fair	Clear, Hot	Calm	Constant
LH	7/21/18	Green	Fair	Clear, Hot	Gusty	Constant
LH	7/29/18	Green	Good	Clear	Calm	Flowing Slow
LH	8/12/18	Green	Good	Cloudy	None	Flowing Slow

Observed measurements in conductivity included fluctuation in all sampled locations (Figure 3.1 C). Conductivity was expected to increase with temperature and salinity. Sudden increases or decreases in conductivity could indicate pollution in the water source. Agricultural runoff can increase conductivity due to introduction of chloride, nitrate, and phosphate ions.[57] Freshwater sources tend to have less pronounced fluctuations in conductivity levels due to long term presence from rock or soil. However, changes in water flow and water levels such as through rainfall or storm events can affect conductivity through their influence on salinity of the water source.[57] Changes in water flow can increase or decrease the conductivity measurements depending on if the water source introduced is highly mineralized groundwater or inflow from another water source of lower salinity. Salinity concentration can decrease by dilution from heavy rainfall.[57] In general, water levels tend to decrease during the summer due to evaporation which results in higher ion concentrated water with increased conductivity values.[57]

Overall, large fluctuations in conductivity occurred between sampling events that are not specifically tied to weather events, with location DJ experiencing a wider range in values than any other location (Figure 3.1 C). During bloom formation, a slight increase in value was observed in conductivity, though conductivity is not reported as directly indicative of algal bloom formations in water sources. Rather, lower conductivity values have been reported to favor blue-green algae growth.[73] Changes in conductivity during algal bloom events can be attributed to correlative variables such as dissolved oxygen, temperature, and salinity. Conductivity shares inverse relationships with dissolved oxygen whereas there are direct relationships with variables temperature

and salinity. KR and Location LR both experienced more dramatic changes in conductivity on the day of location DJ's algal bloom; however, no algal blooms were noted at those locations. Water flow at both locations were constant during this sampling event whereas at Location DJ the water was stagnant. Location DJ consistently had stagnant water flow conditions throughout most sampling events whereas both of these locations varied with flowing slow or constant moving water.

Dissolved oxygen levels also exhibited some variation in measurements between sampling dates. Dissolved oxygen varies inversely with temperature changes throughout the day which is expected due to diurnal variation. Water sources with cooler temperatures tend to have higher dissolved oxygen concentrations and this relationship can be seen through the observed trends amongst sample locations. Only Location DJ experienced a dissolved oxygen level below 6 (mg/L), which corresponded to the time of an algal bloom (Figure 3.1 D). This exceptionally low dissolved oxygen level was expected to be observed during an algal bloom because environments with algal blooms present have anoxic conditions.[74] Blue-green algae development favors these conditions.[75]A simultaneous cycle exists that supplies the water source with hypoxic conditions from depletion of dissolved oxygen while algae decay forms hydroxides that are released back into the water source. This results in decreased dissolved oxygen concentration and increased pH.

3.1.2 Turbidity

Fluctuation in turbidity was expected as turbidity can be influenced by multiple variables including rainfall, water flow, disruption of suspended particles by movement of aquatic wildlife, and bloom formation. Presence of different suspected solids such as

clay, decaying material from algae, or plankton in the water column can affect turbidity values and a turbidity measurement itself does not distinguish between sources of turbidity. [57] The turbidity of Location DJ was of most interest, which saw significant changes in turbidity throughout the summer with an increase of nearly 2 orders of magnitude at the time of a bloom over previous sampling events (Figure 3.2 D). This is expected because stagnant water with no rainfall events (resulting in clear, nonturbid water) is favorable for algae growth. In return, the bloom formation creates a more turbid environment resulting in increased values.[9]

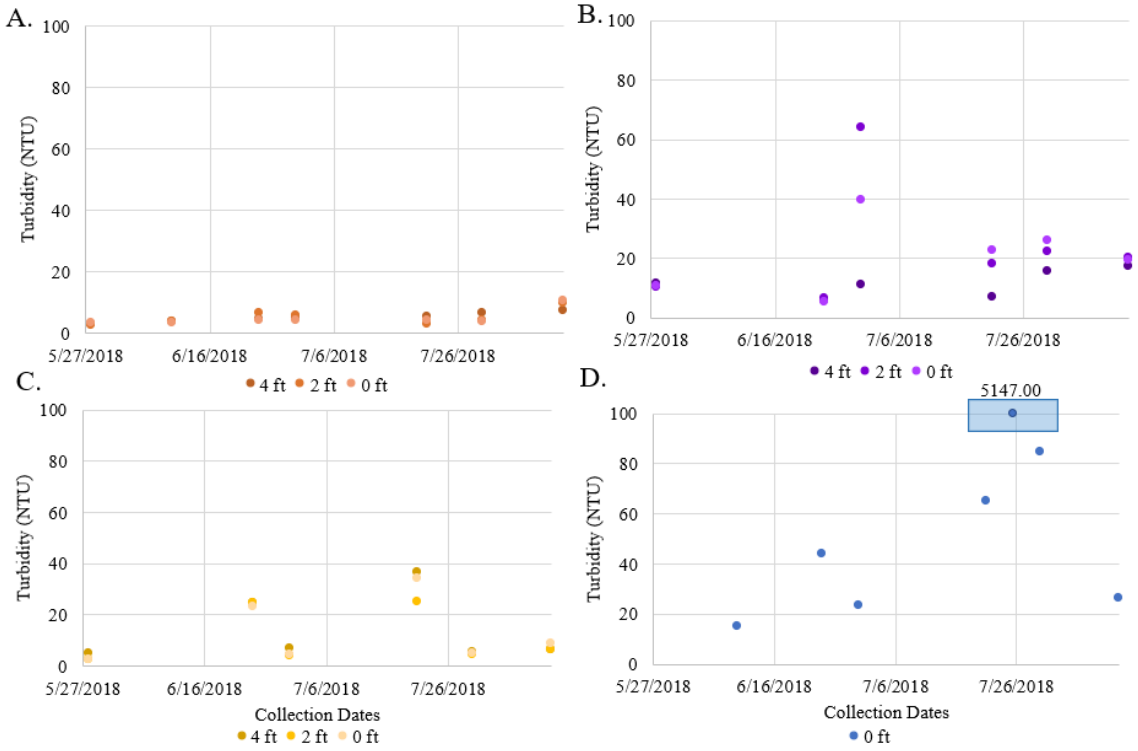

Figure 24 Collected turbidity data for summer 2018 locations including A) Location LH, B) Location KR, C) Location LR, and D) Location DJ.

In non-bloom locations, observed levels of turbidity remained relatively consistent (within 50 NTU) through the course of the summer. Looking at the effect of depth, there were slight variations in values; however, there is no clear trend in turbidity

across the depths. Location KR saw a significant increase during one sampling event (06/30/2018) at surface depth (0 ft) and 2 ft. Weather conditions for this date suggested calm wind and flowing water stirred present sediment more than previous sampling events where water was stagnant. On this day, little sunlight was present as there was overcast during sampling event. There were no visual observations of bloom formations to account for this increased turbidity, though the water was brownish-green color.

3.1.3 Ion Chromatography

The ion chromatography (IC) analysis method was used both summers to evaluate present anion concentrations in water samples. Access to this instrument was made available in late July of summer 2018, so analysis using this method represents a shorter time scale than the environmental monitoring. One aspect that was considered using IC that wasn't represented in the environmental data was the effect of sampling depth on anion levels. Observed anion levels at 2 feet (Figure 3.3) were similar to surface depth and 4 ft depth levels for nitrate, nitrite, and orthophosphate. With these depths, there is a lack of quantifiable levels for such anions. Conclusions cannot be drawn regarding these anions within this depth. For location DJ, measurements were made for surface collection only as this was a shallow water source reaching only between 0.5-1 foot deep. Across all locations, the levels of nitrate, nitrite, and orthophosphate at surface depth, 2 feet, and 4 feet were largely below the limit of quantitation of this method and often below the detection limit of the IC instrumentation.

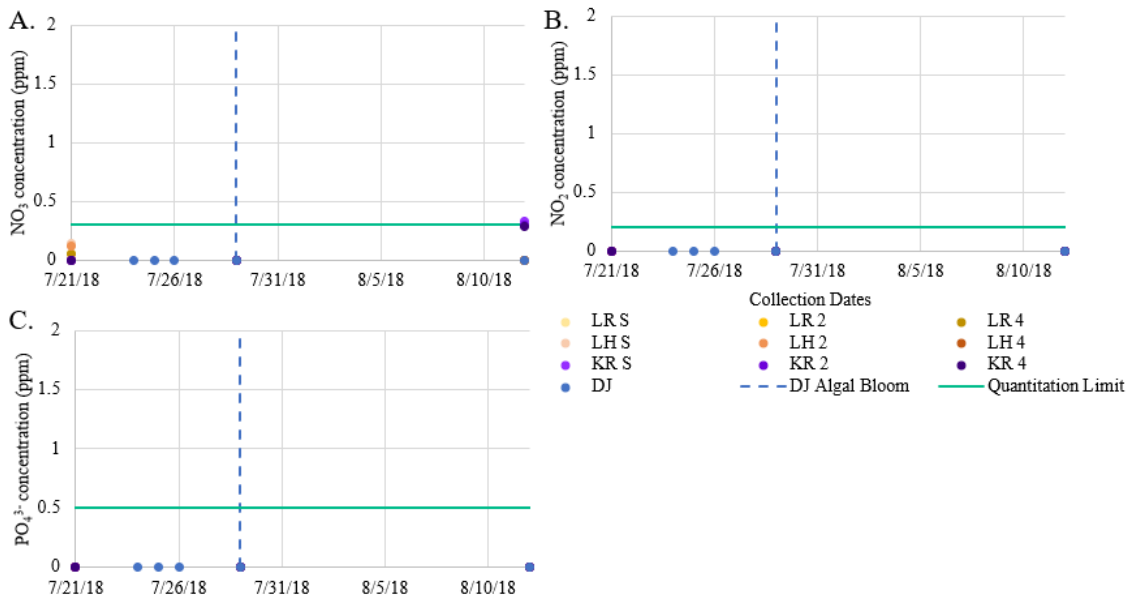

Figure 25 Ion chromatography data for summer 2018 locations at surface depth, 2 feet, and 4 feet including A) nitrate, B) nitrite and C) orthophosphate. The teal line represents the lower limit of quantitation.

Overall data collection revealed no quantifiable amounts of nitrates, nitrites, or phosphates in water samples, contrary to the expected observations. This led to changes in methodology for summer 2019 sampling. Additional methods would be introduced to determine validity of observations from the previous summer. As bloom formations occur most prominently at the surface, this depth was further studied in the following summer to determine clear guidelines of anion levels. In monitoring forms of phosphorous and nitrogen species, high levels of anions were expected during algal bloom events. However, observed levels at all depths throughout the sampling dates were below the technique's limits of quantitation. The quantitation range varied for each anion with nitrate from 0.3 ppm to 20 ppm, and nitrite from 0.2 ppm to 15 ppm, and orthophosphate from 0.5 ppm to 25 ppm. Quantitation limits are represented as solid teal horizontal lines on Figures 3.3 and 3.4.

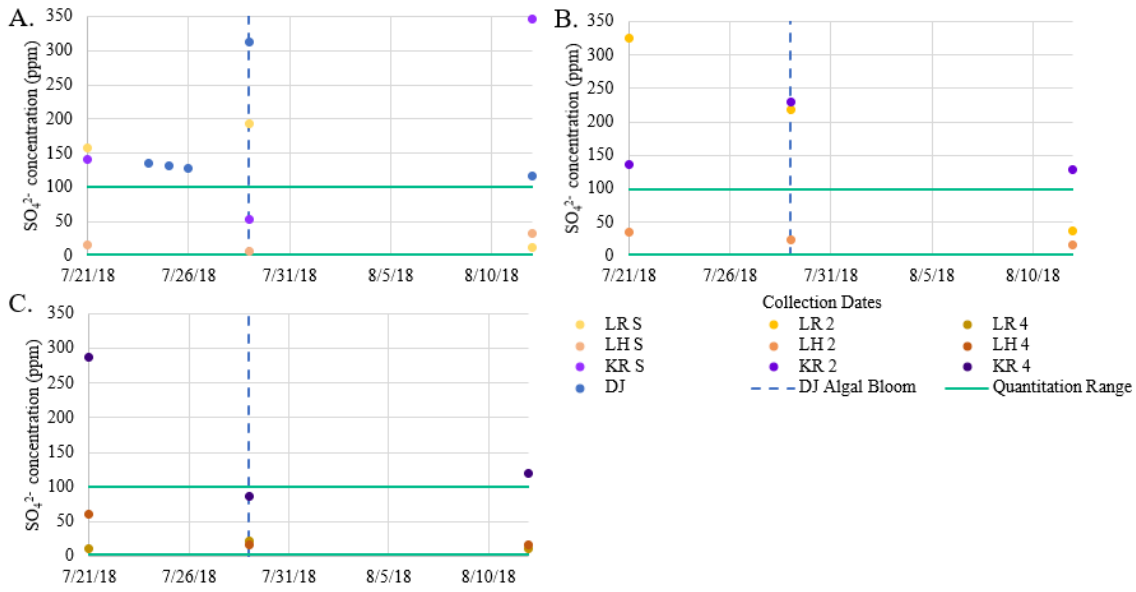

Figure 26 Ion chromatography data for summer 2018 locations for sulfate including A) surface depth, B) 2 feet, and C) 4 feet.

Unlike the other anions, most sulfate levels were within the quantitation range. Location LH measured consistent sulfate levels within the quantitation range (2 ppm to 100 ppm). However, other locations observed levels exceeded the upper limit of the range. While values for these locations are reported, the accuracy of these levels is questionable. Sulfate levels were observed much higher than expected and demonstrated significant variation throughout all locations during different sampling events but did not seem to correlate with times of algae formation. This warranted further investigation into a likely source to determine any causative effects of sulfate presence on algal bloom formation or vice versa.

Additional anions not known for influencing algal bloom formation were also quantified using IC and included bromide, chloride, and fluoride. The results of these analyses can be found in the appendix. All locations indicated low levels of bromide

and fluoride across the time frame of sampling, with most levels below the quantitation limit. Small levels of chloride were present in most locations.

3.2. Summer 2019 Data

Similarly, to Summer 2018, collected data from environmental variables measured at each location site were plotted against time. Since the main focus for this work is identifying conditions that correlate with algal bloom formation, the data presented for summer 2019 sampling will focus on the locations affected by algal blooms. Data for all other locations can be found in the appendix.

3.2.1 Field Observations

Algal blooms were initially visually identified before being confirmed by taxonomic identification using reference guides. Within the 10 locations sampled, three locations were suspected to have at least one algal bloom event with multiple blooms occurring in two of these locations (LR and RH). Harmful algal blooms were observed through distinct field observations including vibrant, prominent colors and a foul odor produced by toxin, though neither scent nor coloration is specific enough for species identification (Figures 3.5-3.8). Viscosity and texture of the blooms were also observed as some blooms are known as being dense and full like paint, whereas others dissipate when in contact with water movement.[61] Microscopic confirmation identified specific algal types for classifying the blooms observed.[60]

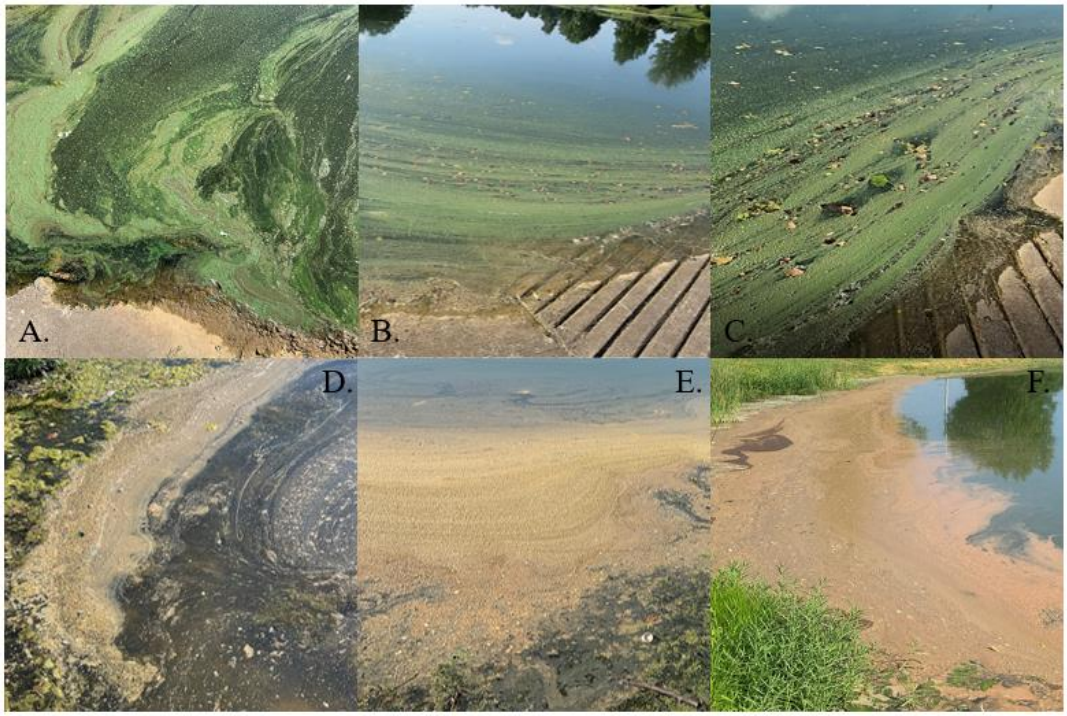

Figure 27 Field images from Lake Reba location (LR) during multiple apparent algal bloom events including those on A) 06/14/2019, B) 07/03/2019, C) 07/05/2019, D) 07/26/2019, E) 07/31/2019, and F) 08/02/2019.

Field images representing the Lake Reba location (LR) algal blooms show the variety of algal growth observed over the summer (Figure 3.5). The LR water source contained multiple bloom events with a harsh odor and viscous texture. This location was expected to have bloom formations due to agricultural runoff influence from surrounding farms and golf courses in the area and has reported blooms occurring in the past few years.[76,77]

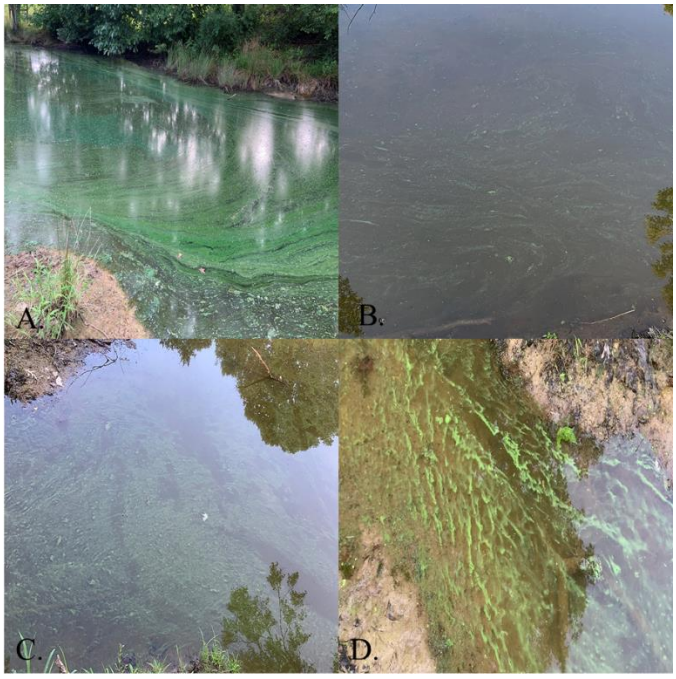

Figure 28 Field images from Rose Anne Harp location during multiple algal bloom events including A) 07/08/2019, B) 07/26/2019, C) 08/02/2019, D) 08/05/2019.

Field images representing Location RH with suspected algal bloom formations shows the variety of observations over the summer (Figure 3.6). This water source contained multiple bloom events with a strong odor but thin viscosity that dissipated with contact. This location was expected to have bloom formations due to agribusiness influences from surrounding farms in the area. This location does not contain agricultural business on its own property, though it is positioned downhill from other active farming sites.

Figure 29 Field image from Tommy Jones location during single algal bloom event during 08/02/2019.

Location TJ observed bloom development on a single date (Figure 3.7). Bloom formations were expected as this water source sits on property with some agricultural influences. Agricultural runoff was expected as it is positioned downhill from farmland containing livestock on the property.

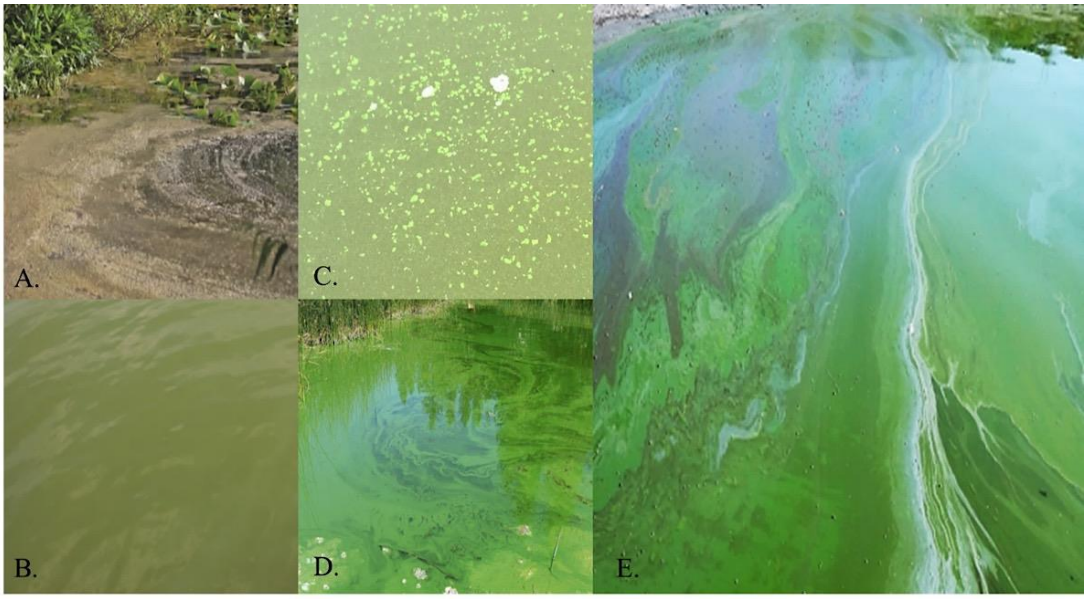

Figure 30 Field imagery from multiple types of harmful algal blooms including A) Cyanobacteria, *Woronichinia*., B) Cyanobacteria, *Microcystis viridis*, C) *Microcystis sp*., D) *Microcystis sp*., and E) mixed CyanoHAB from the Field and Laboratory guide to Freshwater Cyanobacteria Harmful Algal Blooms for Native American and Alaska Native Communities.[60-62]

Classifying bloom formations from field imagery was completed by visual observation using multiple reference guides.[60, 62] Visual characteristics are associated with specific species of algae which is confirmed using microscopy to determine morphology of aquatic microorganisms.[60] Bloom events present in summer 2019 were visually consistent with species of algae including *Microcystis*, *Woronichinia*, Cyanobacteria, *Anabaena*, and mixed genera of multiple CyanoHAB (Figure 3.8). Some field observations indicate the presence of mixed algae where multiple types coexist within the same water source. Water samples containing algae were transported to the lab for taxonomic identification.

3.2.2 Taxonomic Identification

Water samples during algal bloom events were evaluated using taxonomic identification to recognize the characteristics of aquatic organisms. Findings were compared to a phycokey used to describe known identifications of different phylum, genus, and species of algae.[59] Morphological features were used in identifying the presence of microorganisms for bloom classification. Categories used for identification of these organisms include arrangement and shape of cells, attachment onto other microorganisms, presence of sheaths grouped around cells, presence of heterocyst, and pigmentation of enclosed cells.

Identification of algae was based on morphology at the taxonomic level genus. Species classification is not necessary when identifying cyanobacteria unless toxin production is of interest.[61] Water samples containing suspected harmful algal blooms were analyzed the day of collection.

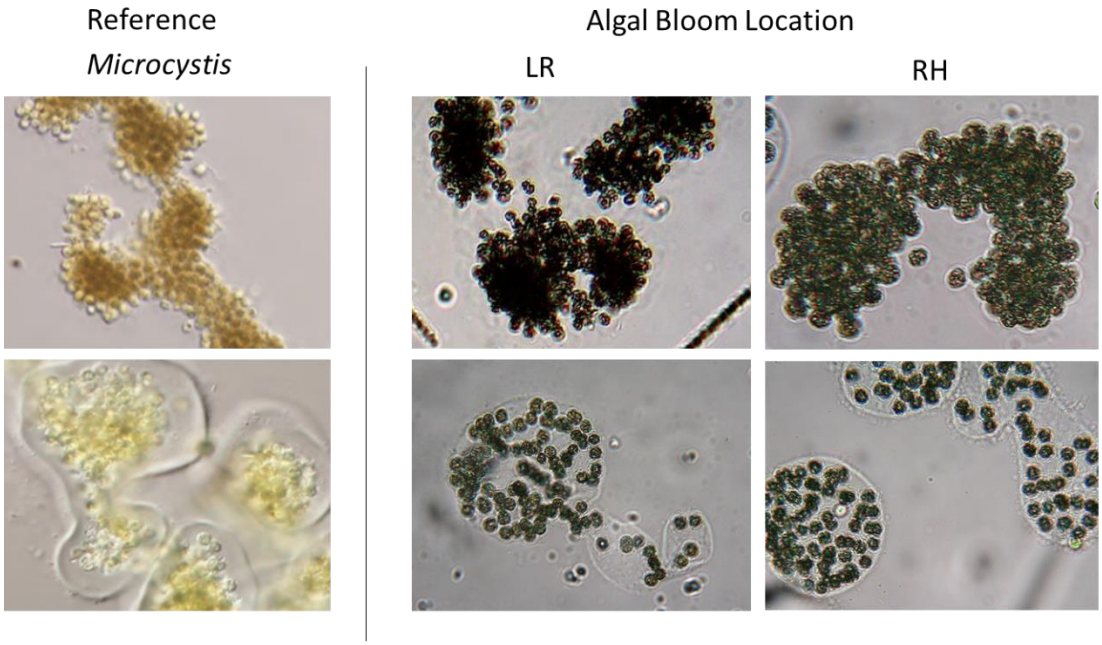

Figure 31 Microscopic images at 40X representing aquatic organism *Microcystis* present during algal bloom events on 06/14/2019 (LR) and 8/02/2019 (RH). Observed results were compared with a Phycokey[60] for organism identification.

Locations LR and RH algal blooms were confirmed to all contain *Microcystins*, the aquatic organisms responsible for production of neurotoxins and hepatotoxins (Figure 3.9). Toxin production originates from *Microcystis aeruginosa*, a species of cyanobacteria that contain conglomerates of algae cells that lack protective sheath layers. *Microcystis aeruginosa* is a species of bacteria that thrives in environments with large phosphorous presence attributed from agricultural or industrial runoff.[78] This bacterium can contain nitrogen storage cells that may be present when the bacterium are starved for nitrogen.

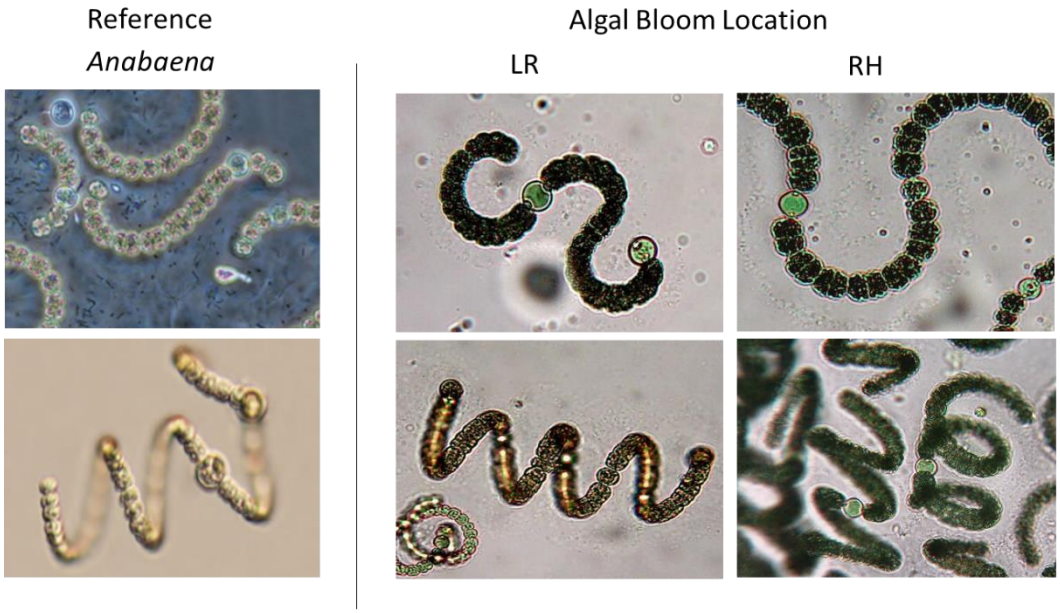

Figure 32 Microscopy images at 40X representing aquatic organism *Anabaena* present during algal bloom events on 06/14/2019 (LR) and 8/02/2019 (RH). Observed results were compared with a Phycokey[60] for organism identification.

Aquatic organism *Anabaena* was observed in both locations. This genus consists of a filamentous cyanobacteria that contains ovoid, light green colored heterocyst areas that developed in limited nitrogen conditions and produce neurotoxins.[79] A string of

vegetative cells makes up the filament backbone to this bacterium that places such organism as a multicellular organism (Figure 3.10). This bacterium is nitrogen fixing in which nitrogen from the air is converted into organic compounds for use of microorganisms. This organism is responsible for production of the neurotoxin anatoxin. Under unfavorable conditions of nutrients, temperature, and saline levels, additional akinete cells are formed.[80]

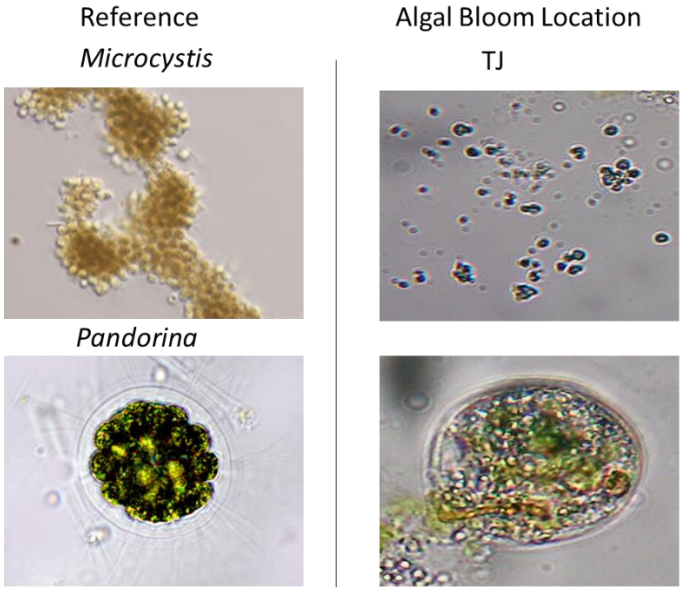

Figure 33 Microscopy images at 40X representing aquatic organisms *Microcystis* and *Pandorina* in TJ location. Observed results were compared with a Phycokey[60] for organism identification.

There was no presence of the *Anabaena* organism in the bloom location TJ, though *Microcystis* cultures were present (Figure 3.11). Colonies of cells had not developed in conglomerates but in unicellular presence. Formation of *Microcystis* consists of small, bright green cells that have been developed though aggregate colonies. An aquatic organism present in all locations was a *Chlorophyceae* genus known as *Pandorina*.[62] This organism is made up of large, ovoid cells varying in brown

or green colors, being tightly packed and surrounded by a thick glue-like wall. This chlorophyte is composed of 8 to 32 cells held together as a colony.

3.3. Environmental Conditions

3.3.1 Expected change versus observed change

There were expected annual trends among some variables including temperature, dissolved oxygen and conductivity. Expected changes were compared to observed changes in collected data from each location. Significant changes in data may result from presence of algal bloom events that distort expected behavior of variables. While the results for all conditions across all sites can be found in the appendix, the results in this section are narrowed to show only the locations affected by algal blooms.

Daily fluctuations in pH were observed by all sampling locations including those that experienced algal blooms (Figure 3.12 A). However, these fluctuations were not considered significant changes as all measured values were between 7 to 9, within the range expected for algal growth. In most cases, when an algal bloom was observed, the pH of that location was observed to increase from the previous sampling date or showed an increase in the sampling date immediately following an observed bloom. This change was expected as the decaying algae release hydroxides into the water sources, causing an increase in pH.

Water temperature at time of collection saw fluctuation in daily sampling events. Expected annual trends were observed for these locations where average temperature increased during the summer months. Most locations gradually increased roughly 5 °C from the original temperature reading during the beginning of the summer (Figure 3.12 B). Sampling times were consistent each day to limit differences in observed

temperature stemming from the daily heating/cooling cycle. Temperature values began to plateau near the end of the summer. Literature reports temperatures reported at greater than 25°C are optimal for algal growth.[21] Temperatures at or above 25°C were observed during most of the sampling events that contained algal blooms. However, Location LR observed a bloom formation in the beginning of the summer when this optimal temperature was not observed, so the literature-reported temperature can be considered an indication of a potential bloom formation but is not a requirement.

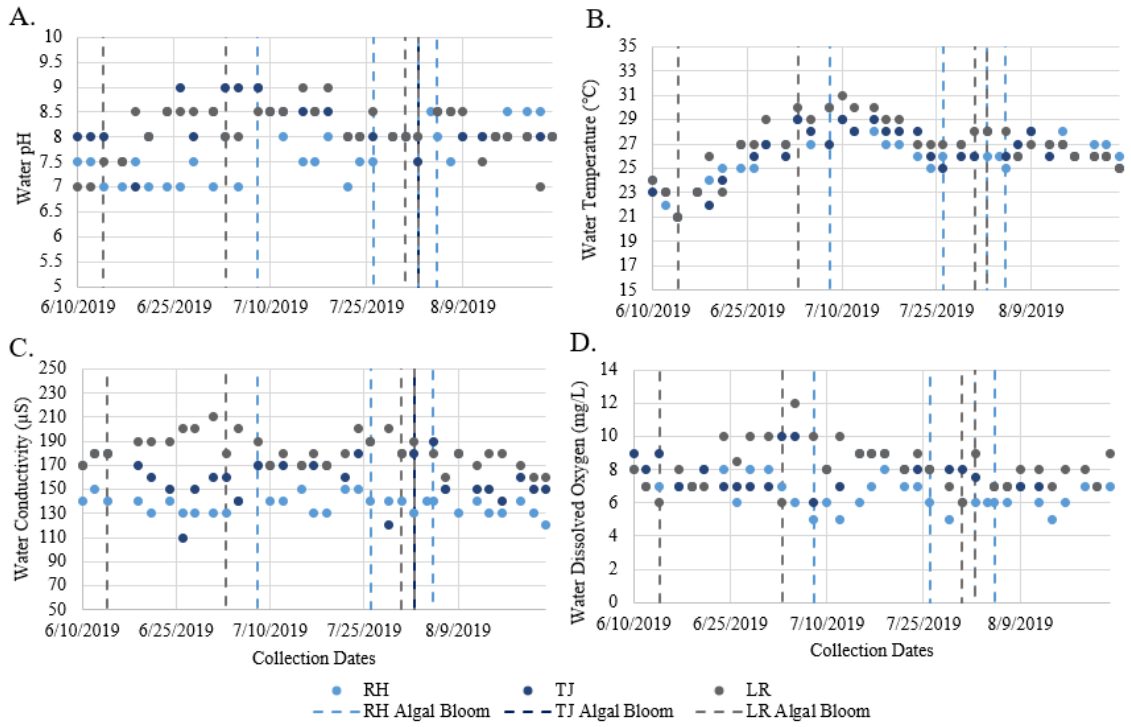

Figure 34 Environmental conditions for summer 2019 locations including A) pH, B) water temperature, C) conductivity, D) dissolved oxygen.

Conductivity measurements observed daily fluctuation with all locations between sampling events. Bloom locations had measurements that varied slightly, though no clear trends in the relationship between algal bloom days and conductivity levels were observed (Figure 3.12 C). Measurements were relatively consistent with

values within a range of 10µS to 30µS between daily measurements with the exception of some dates. Fluctuations in conductivity did not serve as an indicator of bloom formation but rather as an indirect indicator of changes in salinity and temperature. Seasonal shifts in temperature were observed. The higher the water temperature, the higher the conductivity measurements will be as more minerals could be dissolved.[55]

Variation in dissolved oxygen measurements was expected as oxygen levels were affected by numerous variables such as temperature and turbidity. Dissolved oxygen will decrease over the summer as warmer water holds less available oxygen. Additionally, as suspended particles are heated by warming water temperatures, dissolved oxygen levels lower.[81] Fluctuation in dissolved oxygen measurements was also expected due to photosynthesis and respiration. Photosynthesis leads to an expected increase in oxygen levels because of the process releasing more oxygen into the water. Respiration leads to decreased levels in oxygen as the process removed oxygen from the water source. Algal decay leads to an expected increase in oxygen levels due to the influx of hydroxides in water source as algae began to decay. As bloom events occurred, changes in dissolved oxygen included decreases in values leading up to the bloom while after such occurred, values would increase (Figure 3.12 D). Locations affected by algal bloom events observed large variation in values within the range of 5 to 12 mg/L.

3.4 Nutrient Analysis

3.4.1 Phosphorous Analysis

Samples were analyzed for orthophosphate, hydrolysable phosphate, and total phosphate concentration through spectrophotometric methods. Water samples were

analyzed the day of collection, in compliance with federally recommended holding times. Since this technique is based on light absorption, optically turbid samples collected during algal blooms were filtered via vacuum filtration prior to analysis.

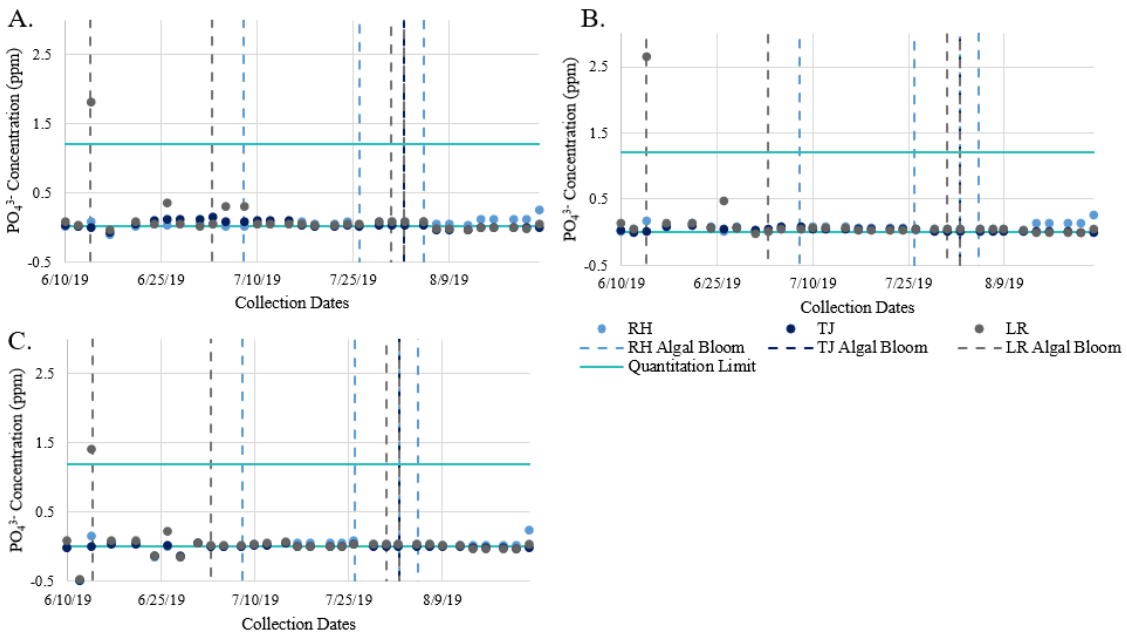

Figure 35 Phosphate analysis using spectrophotometry for algal bloom locations including A) orthophosphate, B) hydrolysable phosphate, and C) total phosphate. The teal line represents the lower and upper bounds of the linear range for this method.

Data collection for orthophosphate testing methods of algal bloom locations observed low concentration values in water samples (Figure 3.13 A). Phosphate analysis for this method has a quantitation range between 0.1 ppm to 1.2 ppm and, while most values fell at or below the low end of this range, there was very little daily fluctuation in the levels of any form. Other forms of phosphate did not see many attainable measurements either, as values remained at the lower limit of quantitation for hydrolyzable and total phosphorous (Figure 3.13 B-C). Measured values were not consistent with disruptions to the environment cause by algal blooms with one exception. It was expected to observe a surge of phosphate levels immediately before or

during an algal bloom event. Location LR observed a large shift in all phosphate concentration of all forms early in the summer during an algal bloom event, driving this measurement to outside of the quantitation range. Other algal bloom events did not observe changes in phosphate concentration. Additional locations observed lower phosphate levels than those affected by bloom events. Little to no fluctuation was observed in concentration levels in these locations. Additional forms of phosphate did not observe measured values different from orthophosphate. Hydrolysable and total phosphorous concentrations remained on the lower limit of quantitation throughout the summer regardless of bloom presence in affected locations. Orthophosphate form was evaluated by additional methods of ion chromatography and semi-quantitative kits using a spectrophotometric method for validation of these low concentration levels.

Possible interferences that ultimately affected measurements of concentrations for the EPA spectrophotometry method includes turbidity. Considering the spectrophotometric technique used, turbid samples were expected to affect measured readings to a greater extent than optically clear samples because of a greater extent of light scattering. While these samples were filtered to minimize the extent of light scattering from suspended, it is apparent this was not a complete correction. For this analysis method, it would be expected to see larger measured values in total phosphorous readings, which should be a summation of all forms of phosphate. However, the observed results received in this study are inconsistent with this expectation. This was a driving factor in the use of additional methods for evaluating phosphate concentrations. As total phosphorous concentrations remain the lowest in measured values, this means these values represent not only the phosphate

concentration but also that turbidity interference that isn't eliminated through filtration prior to analysis. A matrix interference check was conducted to determine if something in the samples were inhibiting analyte concentrations. This interference check was conducted using different techniques including external calibration standards and standard addition. External calibration standards were prepared for this method within the quantitation limits of 0 ppm to 1 ppm. Standard addition involved a known volume of sample water with 1 mL additions of concentrated analyte added to separate flasks. A blank sample and spiked blank sample were also prepared and compared with internal standards analyzed for the same analyte of interest. A pond water sample with no extra analyte of interest added was analyzed against. The figure representing this matrix interference check can be found in the appendix.

3.4.2 Ion Chromatography

Water samples were analyzed for presence of anions of interest including anions reported to be responsible for algal bloom formation such as phosphate, nitrate, nitrite, and sulfate. Additional anions were analyzed including bromide, fluoride, and chloride. External standards were used to monitor accuracy of instrumentation during analysis and for quantitation purposes. The results for locations experiencing a bloom are shown in Figure 3.14 and the results for all locations can be found in the appendix.

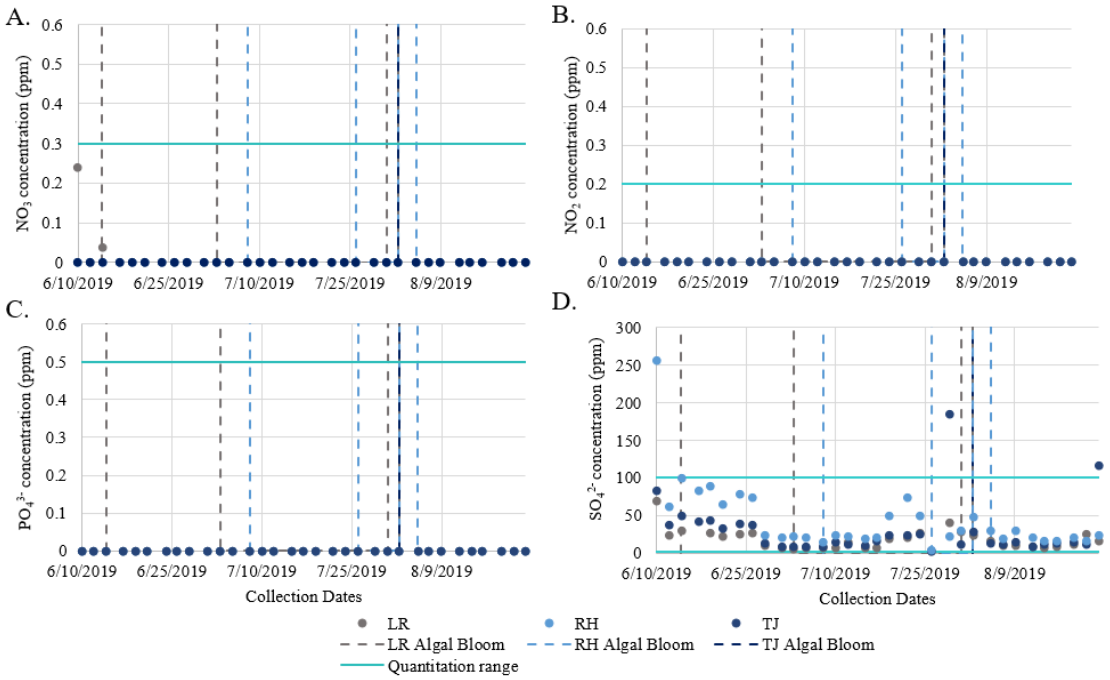

Figure 36 Observed levels for anions of interest by ion chromatography including A) nitrate, B) nitrite, C) orthophosphate, and D) sulfate. The teal line represents the lower limit of quantitation in graphs A, B, and C. The teal line represents the quantitation range with lower and upper limits in graph D.

Based on previous literature, it was expected that the levels of nitrate, nitrite, and orthophosphate would demonstrate a rise in concentration levels during algal bloom events; however, concentrations of all of these ions remained below the analyte's respective limits of quantitation and in most cases below the limits of detection even during algal bloom events (Figure 3.14 A-C). Without quantifiable concentration levels, accurate measurements of any fluctuation in these anion levels could not be determined. Using the spectrophotometric method, orthophosphate levels during an algal bloom event early in June for location LR observed a surge with levels that exceeded the quantitation limits (Figure 3.14 A). However, the IC results here confirmed that the spectrophotometric results were likely affected by matrix interferences (Figure 3.14 C). It should also be noted that the quantitation limits differ for the spectrophotometric and

IC methods, meaning that the ion chromatography method for quantifying phosphate presence does not operate with the same sensitivity and resolution as the spectrophotometry methods. Given the limitations of ion chromatography methods, the quantitation range was within the range of spectrophotometry methods for orthophosphate. Any observations within the spectrophotometry range should have also been observed using ion chromatography. Results between these techniques were expected to be similar.

Observed sulfate levels were primarily seen within the quantitation limits of 2 ppm to 95 ppm for sources experiencing algal blooms, with few days exceeding this limit. Fluctuation of sulfate levels was observed through sampling events with these locations (Figure 3.14 D) but do not appear to clearly correlate with the formation of algal blooms. When examining the underlying cause of high sulfate levels, it was hypothesized that sulfate levels can often be attributed to natural sources originating from rock or sediment within the water source.[82]

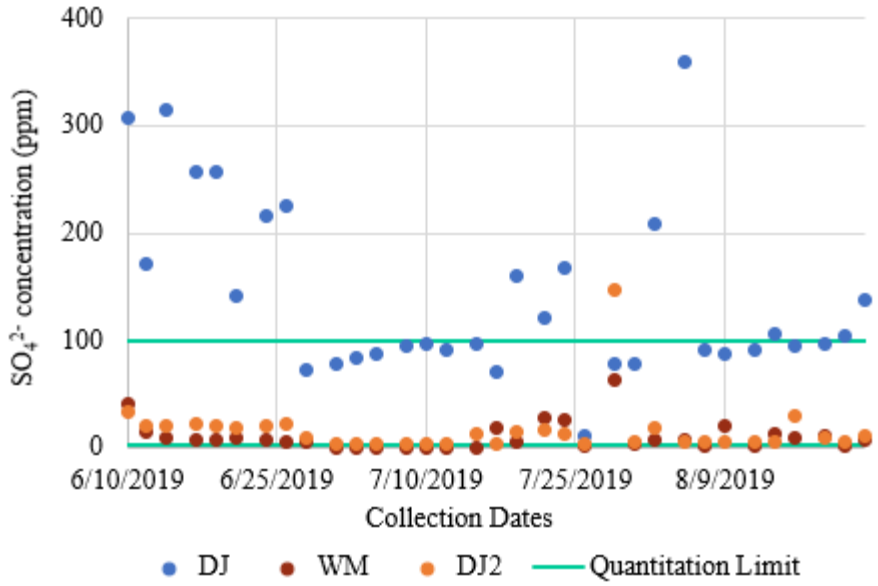

Figure 37 Observed levels for sulfate by ion chromatography for location DJ. The teal line represents the upper and lower limits of quantitation.

To test this hypothesis, the sulfate levels of other locations in close proximity were also observed. This includes locations such as Location DJ, which contained sulfate levels consistently above quantitation limits (Figure 3.15). Though this property didn't experience algal bloom events during summer 2019, it experienced increasingly high sulfate levels compared to other sources and also experienced an algal bloom in summer 2018. This property contains a natural sulfur spring well that flows downhill toward the water source. Other ponds on the property were not assessed in summer 2018; however, high sulfate levels for all locations on the DJ property and in the neighboring property RH were attributed to the natural spring on the property. Additional locations in Waco observed higher sulfate levels than those in Richmond, even when no sulfur spring was in proximity to the source. Higher sulfate levels in these other locations could be attributed to bedrock formations with New Albany Shale, which is known to be present around this area. This bedrock contains pyrite (FeS_2)

which is high in sulfides. Through thermal decomposition and gasification, levels of sulphate are found within ground water.[82]

Location LR also demonstrated high levels of sulfate but is not in as close proximity to the Waco locations. While this can be at least partially attributed to bedrock formation, another possible source is from agricultural runoff that includes sulfate from fertilizer used on surrounding golf courses or farms. However, the exact sources could not be isolated.

Additional anions not known for having a clear influence on algal bloom formation were also monitored including bromide, chloride, and fluoride. All locations recorded little to no presence of bromide and fluoride levels in water samples. If detectable levels were present, they were below quantitation limits for the analytes. Observed chloride levels were seen within quantitation limits ranging from 0.6 ppm to 30 ppm but were generally low in most locations. Observed levels for these anions for all locations can be found in the appendix. Interference checks were conducted with ion chromatography that concluded no inhibition of analyte was determined from matrix interferences. This interference check was conducted using three different samples including an internal standard, spiked blank sample, and a normal pond water sample. An internal standard was prepared with a known volume of pond water, known amount of analyte and diluted to a total volume of 50 mL. A spiked blank sample was also prepared and compared with the internal standard analyzed for the same analyte of interest. A pond water sample with no extra analyte of interest added was analyzed against. The analyte of interest for this interference check was fluoride. The chromatogram representing this check can be found in the appendix.

3.4.3 Turbidity

Observed data showed fluctuation in turbidity throughout the summer, as expected with changes in water flow, stirred sediment, and algae growth (Figure 3.16). The most extreme changes in turbidity were observed just before or during algal bloom events. These values provide quantitative measurements for the visual observations of high-water turbidity surrounding an algal bloom. Little fluctuation in measurements was observed for days not experiencing bloom events, correlating with the visual observations of clear water. Surges in turbidity mainly correlated with confirmed algal bloom events for these locations; however, there were days with increased turbidity though no bloom was confirmed. Location RH saw increases in turbidity leading up to algal bloom events. As conditions were becoming favorable for bloom formation, surges in turbidity could be observed. Most days with confirmed bloom events saw evident spikes to higher turbidity values. Location LR saw a day with higher turbidity values though no bloom was confirmed (Figure 3.16). On June 26th development of thin brown film was observed on the surface of the water which was collected for confirmation of bloom. However, taxonomic identification confirmed no microorganisms associated with HABs were present. In addition to algal bloom formation, weather conditions can lead to turbid water. Most weather conditions surrounding the June 26th sampling were compatible with harmful algae formation including optimal water temperature (at or above 27°C), some rainfall the day before this sampling event, and direct sunlight with no overcast of clouds on the day of bloom.

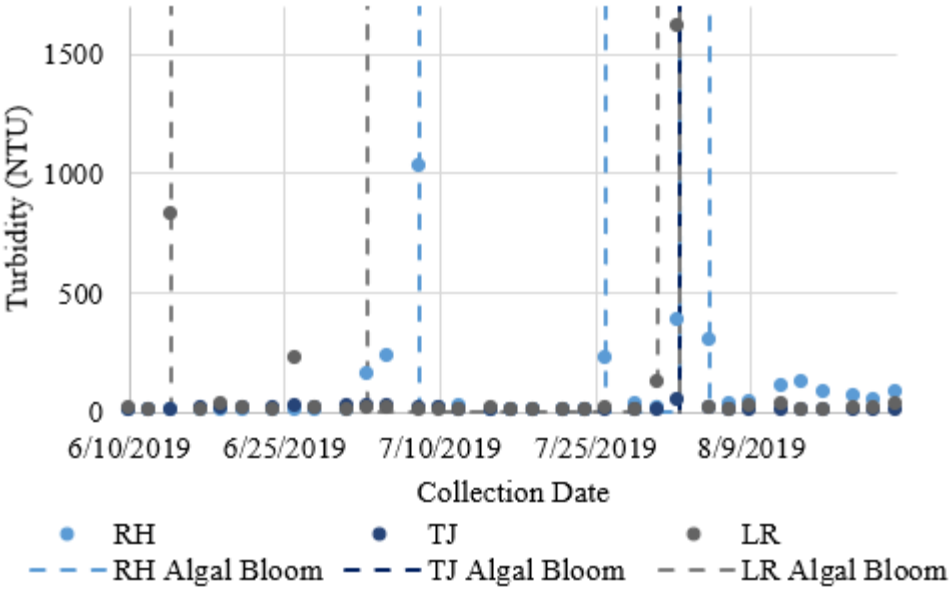

Figure 38 Observed turbidity data for summer 2019 sampling events for algal bloom locations.

Some conditions did not favor bloom formation including higher dissolved oxygen measurements of 8.5 mg/L on this day and 10 mg/L on the sampling event prior to this sampling event. It is then likely that full bloom development did not occur for this water source due to unfavorable conditions of dissolved oxygen and rainfall. This rainfall event resulted in higher turbidity measurements as the water stirred the sediment. All other conditions besides dissolved oxygen were favorable for this water source to have a harmful algal bloom event. Increased measurements on this day are attributed to prior rainfall events. Turbidity data for locations not affected by bloom events can be found in the appendix but showed no extreme changes in turbidity throughout the summer.

3.5 Semi-Quantitative Analysis of Anions

The use of additional semi-quantitative testing methods for phosphate, nitrate, nitrite, and sulfate served two main purposes. First, testing kits were used to confirm the trends observed using the spectrophotometric phosphate analysis and the IC analysis, specifically confirming the low phosphate, nitrate and nitrite concentrations and the high sulfate concentrations. Second, the commercially available testing kits were used to establish the level of quantitative data available for lay persons monitoring their private water sources. Selective locations were chosen for this study to include locations affected by algal bloom events, locations with agribusiness influences on the property, and locations without any presence of nutrient loading on the property.

3.5.1 Phosphates

Observed phosphate levels using the semi-quantitative kits were lower in concentration than those proposed by analytical techniques (Figure 3.17), however values obtained from the kits were within the recommended concentration range for the technique. It was expected for all methodologies (IC, spectrophotometry, and semi-quantitative kit) to have similar results during normal conditions and bloom conditions. Observed testing kit results saw similar lower concentrations as was seen with spectrophotometry methods although results were not consistent with algal bloom formations. These testing kits were expected to observe increases in concentration during algal bloom events, though this was not the case. No changes were observed during algal bloom events for orthophosphate concentration while using the semiquantitative kits. Observed levels were within quantitation limits but hung near the lower limit of this range. Some levels were below this limit and couldn't be accurately

quantified. Attainable results could not be compared with ion chromatography methods due to differences in quantitation ranges. Ion chromatography has a higher quantitation range whereas spectrophotometry is more likely to overlap with testing kit methods linear range. These testing kits were expected to replicate instrumentation results so the lay person could assess their water source accurately. This testing kit could not be used to assess water quality specifically for algal bloom formation. Samples were not filtered in testing kit methods to duplicate available testing procedures of lay persons in the field.

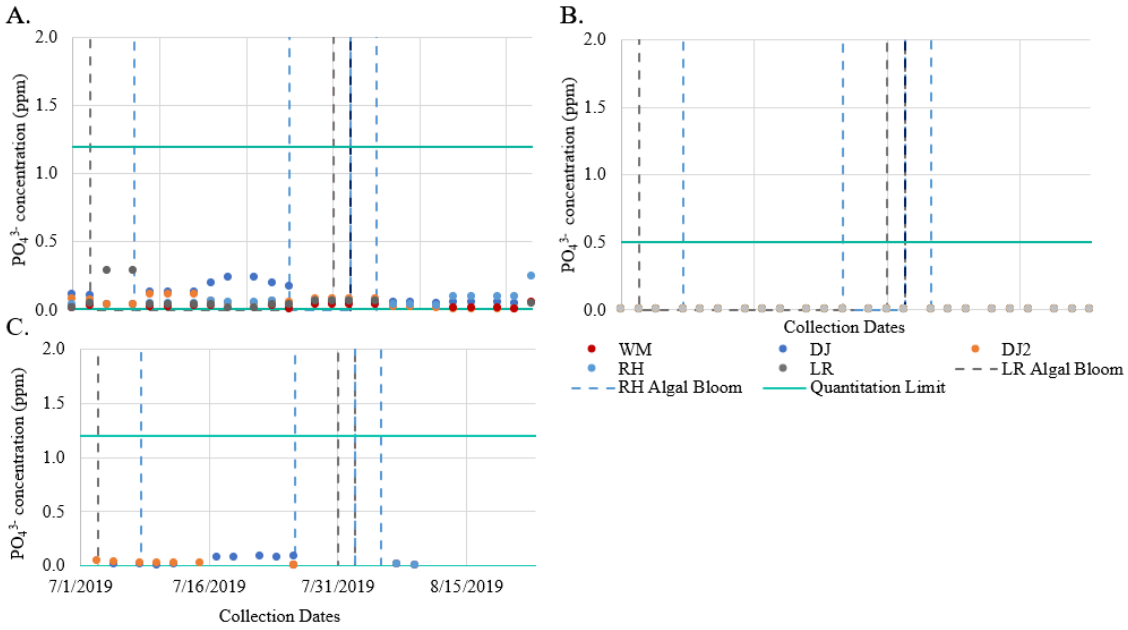

Figure 39 Orthophosphate concentrations differentiated through additional testing methods including A) spectrophotometry, B) ion chromatography, C) semi-quantitative testing kit. The teal line represents the upper and lower limits for graphs A and C for the quantitation ranges of these methods. For graph B, it represents the lower limit of quantitation.

3.5.2 Nitrates

Observed nitrate levels were higher in concentration using the semi-quantitative kits than those the levels determined by ion chromatography (Figure 3.18). Expectations

were to see similar observed results for both analytical methods and semi-quantitative testing kits. The IC method resulted in little to no presence of nitrates present in water sources whereas testing kits saw large fluctuations in concentration.

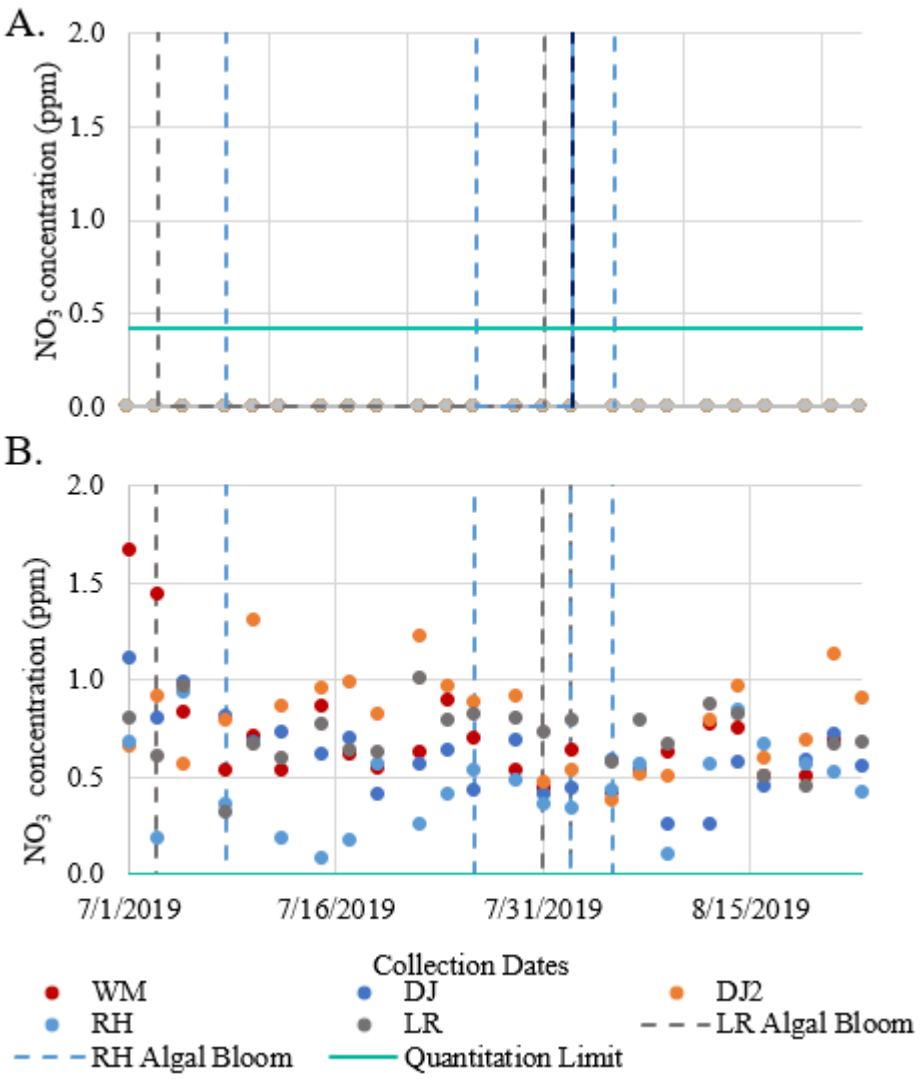

Figure 40 Nitrate concentrations differentiated through additional testing methods including A) ion chromatography and B) semi-quantitative testing kit. The teal line represents the lower limit of the quantitation range for these methods.

Surges in nitrate were expected during algal blooms though this was not the case. Though there were no similarities between acquired results of both methods, observed results from testing kits were not consistent with bloom events. Quantitation

ranges of each method differ, although both methods share some overlap within the linear range. Those results saw large fluctuations in data between sampling events. Samples were not filtered in testing kit methods to replicate available testing procedures of lay persons in the field so the marked differences between the methods may be due to the presence of matrix interferences. These interferences can be problematic for spectrophotometric methods and result in higher measured values for nitrates. This semi-quantitative testing kit would not be acceptable for prediction of algal bloom formation as results could not be accurately validated with additional testing methods.

3.5.3 Nitrites

Observed nitrite levels were higher in concentration (Figure 3.19 B) than those proposed by analytical techniques. Expectations were to see similar observed results for both analytical methods and semi-quantitative testing kits. Analytical techniques resulted in little to no presence of nitrites present in water sources whereas testing kits saw large fluctuations in concentration. Surges in nitrite were expected during algal blooms though this was not the case. Quantitation ranges of each method differ, although both methods share some overlap within the linear range. Those results saw large fluctuations in data between sampling events. Observed results included some concentrations within the quantitation range and some below it. Regardless, attainable results were not consistent with analytical methods and could not be validated. Interferences from turbid water samples showed to have an influence over measured readings for analyte concentrations acquired using spectrophotometry methods. Additional analysis methods using ion chromatography did not share similar results in analyte concentrations. Semi-quantitative testing kits could not be accurately validated

for laboratory use in quantifying concentrations. These testing kits could not be concluded as validated for use in the field by lay persons to predict algal bloom formations.

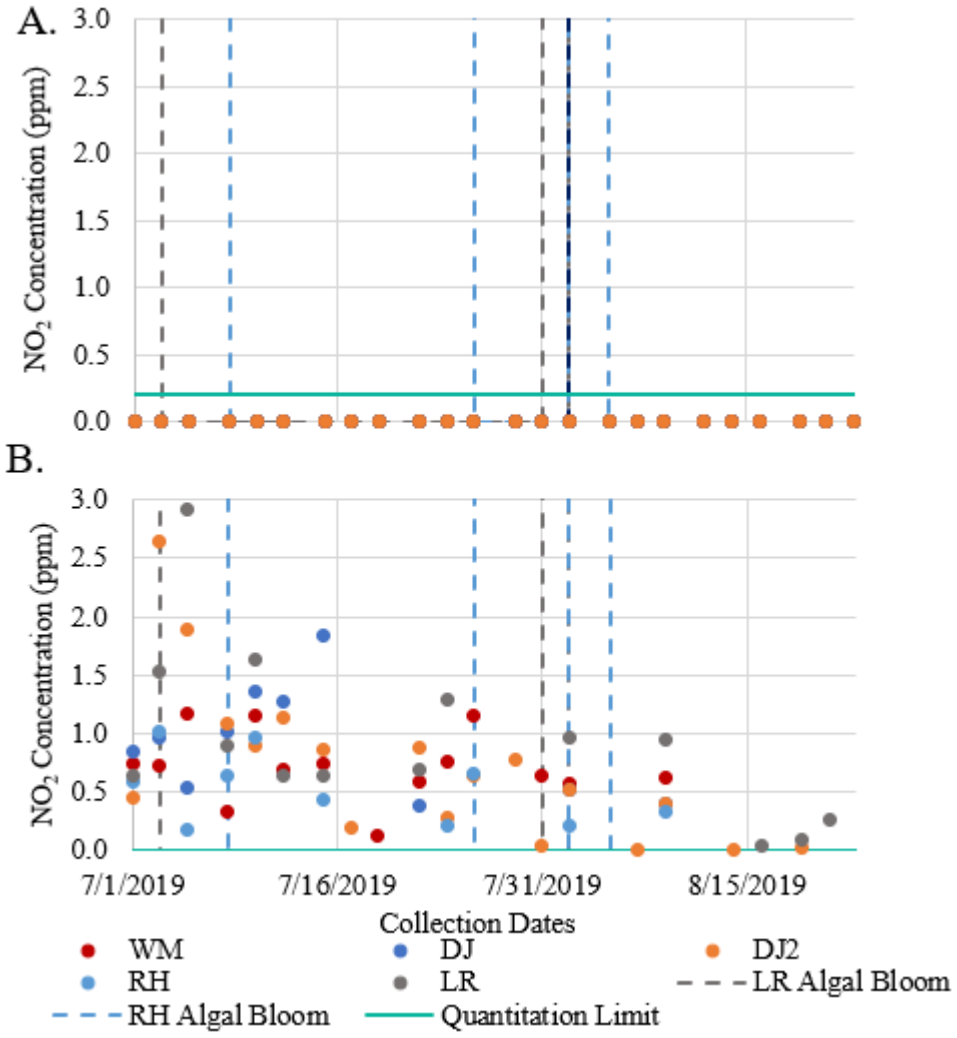

Figure 41 Nitrite concentrations differentiated through additional testing methods including A) ion chromatography and B) semi-quantitative testing kit. The teal line represents the lower limit of the quantitation range for these methods.

3.5.4 Sulfates

Additional testing methods to quantify sulfate concentration in water samples through ion chromatography methods and testing kit methods. Similar results were

expected using analytical techniques and testing kit methods. Observed levels of sulfate were high in concentration. Sulfate levels were found within quantitation limits of testing kit methods (Figure 3.20 B). Fluctuation was observed between sampling events for select locations DJ and RH. Little to no fluctuation was observed for additional locations. Higher sulfate concentrations were observed for these locations, though levels observed were not consistent with algal bloom events. Due to limiting reagents, selective days were analyzed for sulfate levels using testing kit methods. Ion chromatography methods observed high concentrations in sulfate. Some locations could not be quantified as observed levels exceed quantitation limits for this method. Testing kit methods had a larger quantitation limit, though results were not similar between analytical methods. Significant changes in sulfate concentrations were observed using testing kit methods prior to or during algal bloom events for affected locations. Similar results were not attained using ion chromatography methods and the results were not consistent with algal bloom formations.

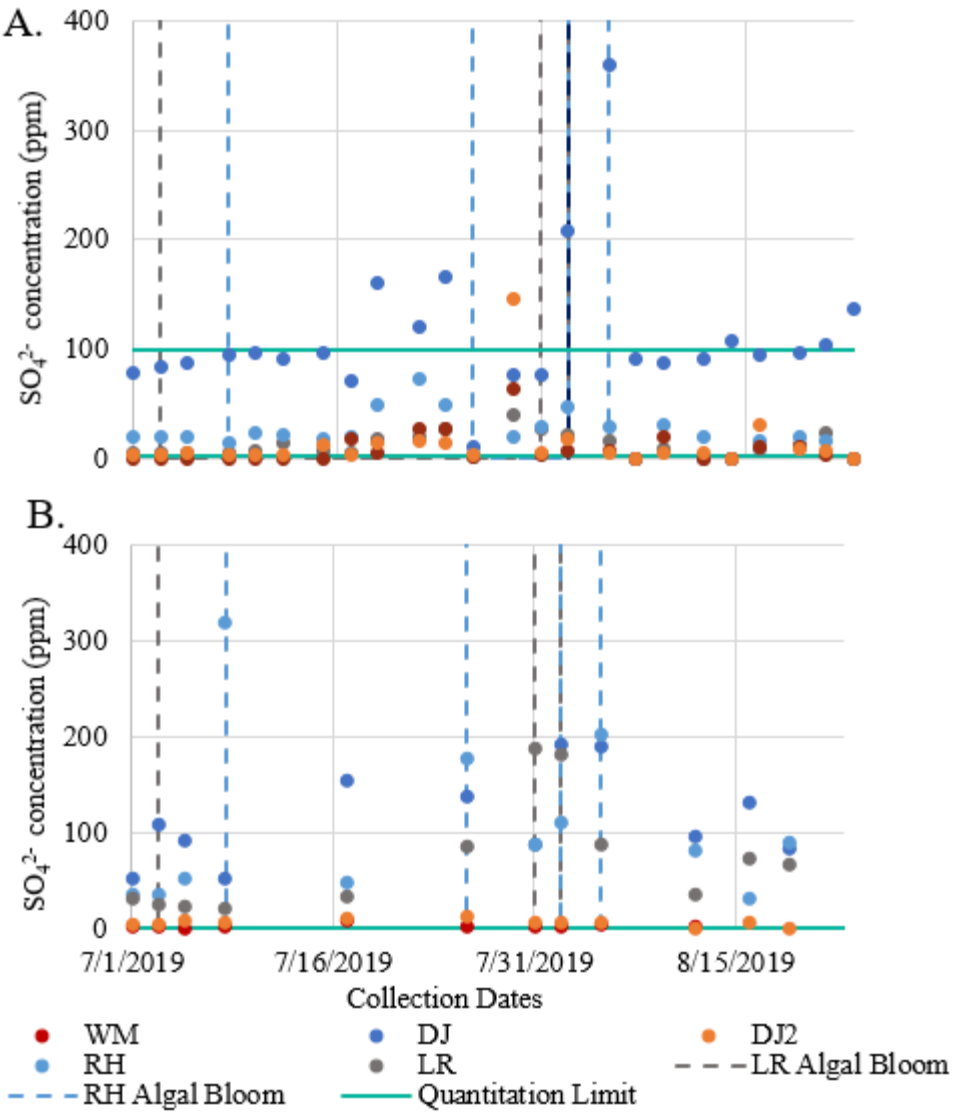

Figure 42 Sulfate concentrations differentiated through additional testing methods including A) ion chromatography and B) semi-quantitative testing kit.

Samples were not filtered in semi-quantitative testing to replicate available testing methods of lay persons in the field, which means that interferences from turbid samples likely affected measured concentrations of sulfate in water samples. The high starting turbidity of the sample resulted in higher concentrations of NTU being recorded during the sulfate test and ultimately correlates to a higher sulfate level being reported than was actually present.

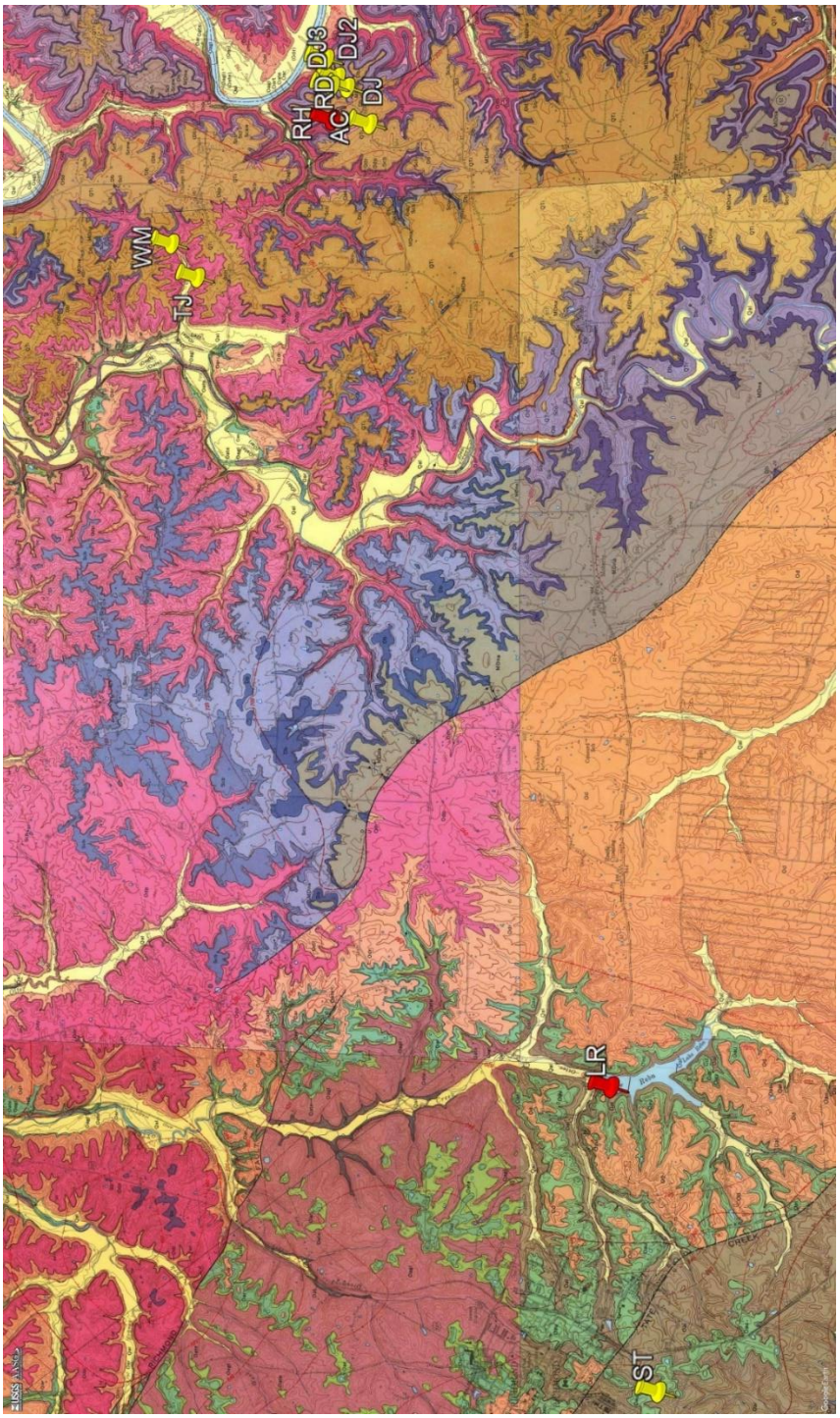

Figure 43 Thermal map containing all locations using Google Earth. Various types of bedrock are established using different colors associated by composition. Locations affected by algal blooms are marked as red thumbtacks. Locations not affected by blooms are labeled as yellow thumbtacks.[83]

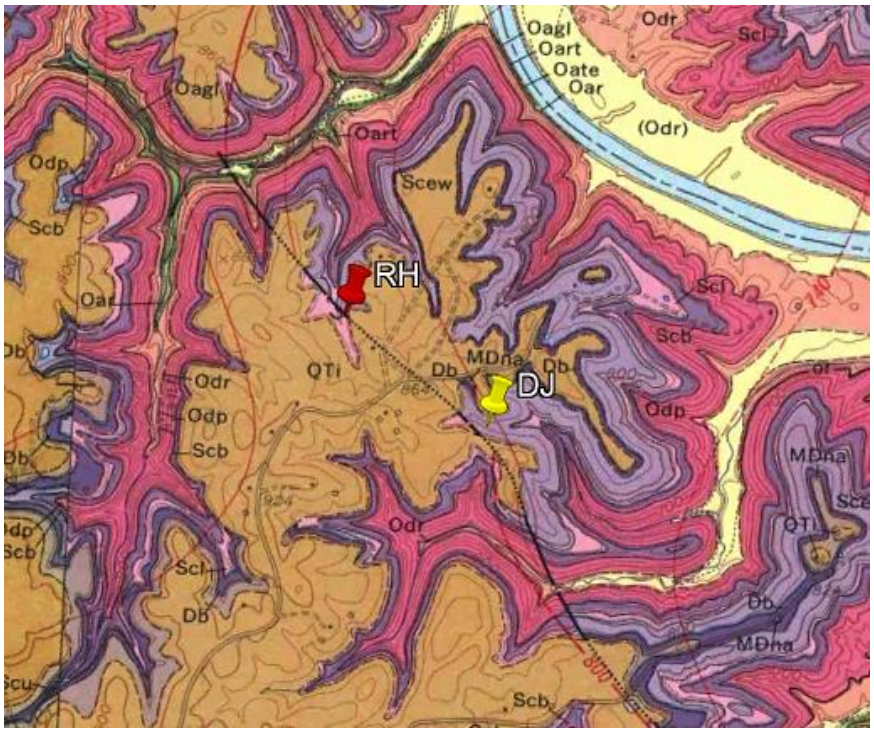

Figure 44 Magnified Palmer map of DJ and RH locations using a thermal filter. Various types of bedrock are established using different colors associated by composition. Shades of purple represent New Albany Shale labeled as MDna.[83]

Sulfate concentration was found to be present in most locations but did not contribute to algal bloom events. Though one location's high levels of sulfate was connected to presence of a natural spring, it does not explain the high sulfate levels for other areas. Investigating the potential of the bedrock and sediment as a source of the sulfate, it was determined that the sampled locations sit on top of bedrock that contained New Albany Shale (Figure 3.22). US Geological Maps of the palmer quadrangle are used in determining the bedrock formation for these locations.[83] This bedrock contains various minerals including pyrite, which contains iron sulfide (FeS_2). Through pyrite oxidation water is introduced where it reacts to form sulfate and ion oxyhydroxides.[84] In the weathering of pyrite, it is oxidized by oxygen to form sulfate and ferrous iron.

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+$$ Equation 3.25**.**

Locations LR, DJ, and RH do not share property lines, though these sampled locations contain higher sulfate levels than others selected locations. Though all locations sit on bedrock with some influence of mineral composition, locations found near DJ and RH were expected to have similar sulfate levels, but this was not observed. Bedrock formation within each location likely contain different composition which could have influenced each locations level differently. Location RH and DJ can be found surrounded by bedrock labeled as Irvine formation (QTI) and New Albany Shale (MDna). Irvine formation is consisted of various types of silt, clay, sand, and sandstone consistent with pyrite. Through processes of erosion and weathering, sulfur presence could be influenced by groundwater contamination from said pyrite presence within such bedrock amongst locations.

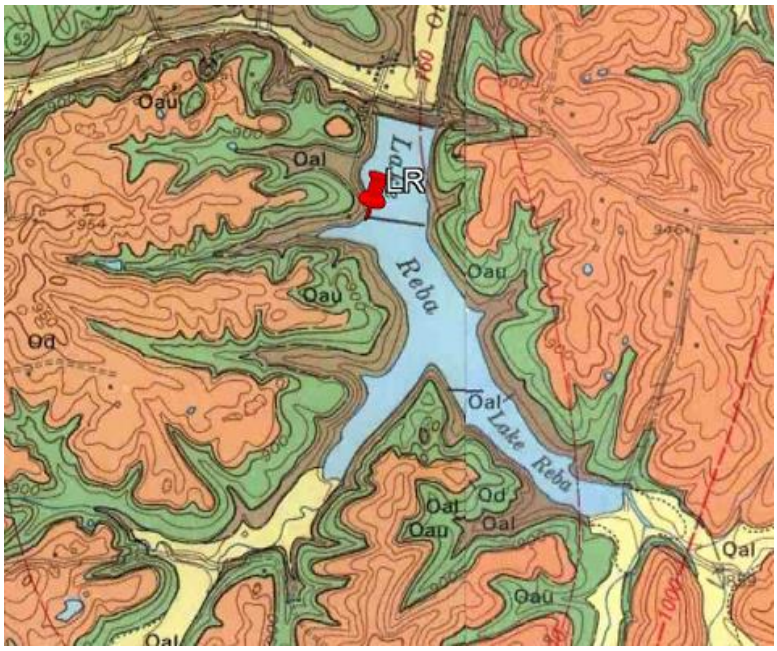

Figure 45 Magnified Quadrangle map of LR location using a thermal filter. Various types of bedrock are established using different colors associated by composition.[83]

Location LR can be found surrounded by bedrock labeled as OAU and OAL. This formation is known as Ashlock which consists heavily of limestone and dolomite.

There are large surrounding areas made up of Drakes formation or OD. This contains large amounts of limestone, dolomite, and shale.[83] Limestone and dolomite are minerals contained in rock formations known as composed of calcium carbonate or calcium magnesium carbonate. Shale is composed of a mix of clay minerals such as quarts or calcite. This bedrock combination does not contain pyrite. Higher sulfate levels are likely associated with agricultural runoff attributed to fertilizer use for turf from surrounding golf courses on the property.

3.6. E. coli

All locations were analyzed using *E. coli* testing methods to determine influence of its presence on algal bloom events. Suspected influence of bacteria presence was further investigated to determine effects on algal bloom events. Locations were analyzed during select dates based on instrumentation availability. Results from *E. coli* testing methods is in the form of most probable number values (MPN), which are values that are associated with number of colonies present in 100 mL of sampled water. Values were placed into categorial gradients based on standard guidelines of Kentucky Watershed Watch and the Kentucky Geological Survey. State criteria for *E. coli* guidelines is based on use of water source while being set by sampling frequency, reporting results as “chronic” for a value of 130 MPN per 100 mL, “acute” for 240 MPN per 100 mL, and “very high” for 2400 MPN 100 mL.[85]

Date	Sample	E COLI MPN/100 mL	T. COLIFORM MPN/100 mL	Date	Sample	E COLI MPN/100 mL	T. COLIFORM MPN/100 mL
6/20/2019	AC	325.7	437.4	8/20/2019	RD	2	2419.6
7/4/2019	AC	2419.6	2419.6	8/22/2019	RD	1	5.2
8/17/2019	AC	1	2419.6	8/24/2019	RD	1	2419.6
8/20/2019	AC	6.3	456.9	6/20/2019	RH	437.4	2419.6
8/22/2019	AC	1	93.3	7/4/2019	RH	2419.6	2419.6
8/24/2019	AC	1	2419.6	7/9/2019	RH	2419.6	2419.6
7/4/2019	DJ	2419.6	2419.6	8/17/2019	RH	24.6	1986.3
8/17/2019	DJ	12.1	217.2	8/20/2019	RH	38.4	2419.6
8/20/2019	DJ	104.6	2419.6	8/22/2019	RH	22.8	2419.6
8/22/2019	DJ	1732.9	2419.6	8/24/2019	RH	14.6	241.1
8/24/2019	DJ	12.1	1986.3	6/20/2019	ST	241.1	2419.6
7/4/2019	DJ2	2419.6	2419.6	7/4/2019	ST	2419.6	2419.6
8/17/2019	DJ2	1986.3	2419.6	8/17/2019	ST	1986.3	2419.6
8/20/2019	DJ2	1	2419.6	8/20/2019	ST	161.6	1011.2
8/22/2019	DJ2	3.1	2419.6	8/22/2019	ST	21.6	2419.6
8/24/2019	DJ2	533.5	2419.6	8/24/2019	ST	1986.3	2419.6
8/17/2019	DJ3	1011.2	65	6/20/2019	TJ	164.4	691
8/20/2019	DJ3	1	228.2	8/17/2019	TJ	3.1	2419.6
8/22/2019	DJ3	1	1011.2	8/20/2019	TJ	8.6	2419.6
8/24/2019	DJ3	1	2419.6	8/22/2019	TJ	13.4	2419.6
8/17/2019	LR	461.1	72.8	8/24/2019	TJ	3.1	2419.6
8/20/2019	LR	45	2419.6	6/20/2019	WM	223	2419.6
8/22/2019	LR	146.7	2419.6	7/4/2019	WM	691	2419.6
8/24/2019	LR	461.1	1203.3	8/17/2019	WM	3.1	2419.6
6/20/2019	RD	90.7	1011.2	8/20/2019	WM	3.1	387.3
7/4/2019	RD	87.8	2419.6	8/22/2019	WM	40.4	172.2
8/17/2019	RD	1	290.9	8/24/2019	WM	3.1	1553.1

Figure 46 MPN values for select dates of locations evaluated using *E. coli* testing methods. Levels of E. coli are represented as colored boxes of green for "chronic", yellow for "acute", and red for "very high".

Select dates were evaluated due to limited availability to instrumentation. Some dates were evaluated near algal bloom events for select locations while others were evaluated to assess water quality. Increases in MPN results were expected to be observed during algal bloom events. Fluctuation of *E. coli* data points could be affected by daily fluctuation of bacteria presence. Locations affected by algal bloom events such as RH saw increased MPN values from acute to very high, though selected days of analysis were over 2 weeks apart and did not include bloom presence. Values remained

very high during the course of a bloom event, though values could not be accurately attribute to bloom formation or other variables at play before these dates. Additional testing could improve this testing method to assess importance of E. coli presence during algal blooms. Location LR was monitored near the end of the summer, though observed levels of *E. coli* were found between low values with some fluctuation to acute levels. Significant conclusions can't be made for *E. coli* observations attributing to algal bloom events due to lack of sampling point frequency and instrumentation availability.

3.7. Multivariate Analysis

3.7.1. Data Tables

Conditions expected during algal bloom formations included very low dissolved oxygen, higher water temperatures, and high levels of nutrients. However, the observed data did not see obvious trends in dissolved oxygen that were consistent with algal bloom formations for affected locations. The strongest correlation observed involved turbidity measurements. Nutrients were not able to be quantified at the levels that were present in the water sources throughout the summer. Statistical analysis was performed to tease out trends in observed data to establish the contributable variables during algal bloom formations. It was expected to see locations cluster by similar data trends using PCA. Obvious outliers would be investigated to determine what variable contributed to said distance from clustered location. Additional data tables for all locations can be found in the appendix.

Table 47 Data table for location "RH" including classification, environmental conditions, and in lab data.

Sample Site	Rose Anne Harp Property
Sample ID	"RH"
City Location	Waco
Latitude	37.767837°
Longitude	-84.114238°
Water source type	Private reservoir
Influences	Agricultural (surrounded by agribusiness properties)

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	74	7.5	24	140	8	0	10:14	10:20	1
6/12/19	N	60	7.5	22	150	7	0	8:50	9:00	0.25
6/14/19	N	50	7	21	140	7	0	8:41	8:45	0
6/17/19	N	72	7	23	**N/A	7	1	8:50	8:58	0.4
6/19/19	N	69	7.5	24	140	7	1	7:26	7:34	0.3
6/21/19	N	71	7	25	130	8	2	8:32	8:38	0.45
6/24/19	N	72	7	25	140	8	1	8:31	8:37	0.5
6/26/19	N	66	7	25	130	6	1	8:21	8:29	0
6/28/19	N	69	7.5	27	130	8	1	8:30	8:35	0
7/1/19	N	70	7	27	130	8	1	8:31	8:40	0
7/3/19	Y	73	8	29	130	7	1	8:55	9:05	0.2
7/5/19	N	70	7	27	140	6	2	8:26	8:36	0
7/8/19	Y	72	9	30	170	5	3	8:46	8:55	0.35
7/10/19	N	74	8.5	29	140	6	2	8:16	8:22	0
7/12/19	N	69	8	28	140	5	2	8:29	8:35	2
7/15/19	N	73	7.5	28	150	6	2	8:36	8:45	0.8
7/17/19	N	74	7.5	27	130	7	1	8:32	8:37	0.25
7/19/19	N	75	8	27	130	8	2	8:30	8:35	0
7/22/19	N	73	7	26	150	7	1	8:27	8:39	0.5
7/24/19	N	59	7.5	25	150	7	1	8:26	8:34	0
7/26/19	Y	62	7.5	26	140	6	3	8:16	8:33	0
7/29/19	N	72	8	27	140	5	3	9:30	9:35	0
7/31/19	Y	67	8	26	140	6	2	8:30	8:35	1.2
8/2/19	Y	67	8	26	130	6	2	7:16	7:24	0
8/5/19	N	69	8.5	26	140	6	2	8:18	8:25	0
8/7/19	Y	63	8	25	140	6	2	8:30	8:35	0
8/9/19	N	68	7.5	26	150	6	2	8:30	8:38	0
8/12/19	N	71	8	28	130	7	2	8:26	8:35	0
8/14/19	N	70	8	27	140	6	2	8:24	8:35	0
8/16/19	N	73	8	28	130	5	2	8:20	8:28	0.3
8/19/19	N	66	8.5	26	130	6	2	8:28	8:35	0.1
8/21/19	N	70	8.5	27	140	7	1	8:40	8:46	0.2
8/23/19	N	71	8.5	27	130	7	1	8:31	8:37	0
**June 17th, conductivity measurement not taken due to unavailable instrumentation.										

Table 48 (continued).

Sample Site	Rose Anne Harp Property
Sample ID	"RH"
City Location	Waco
Latitude	37.767837°
Longitude	-84.114238°
Water source type	Private reservoir
Influences	Agricultural (surrounded by agribusiness properties)

		Turbidity	Ion Chromatography							Spectroscopy
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	4.75	0.00	3.14	0.00	0.00	0.00	0.00	254.46	0.011
6/12/19	N	4.01	0.00	3.23	0.00	0.00	0.00	0.00	61.14	0.004
6/14/19	N	3.85	0.00	2.47	0.00	0.00	0.00	0.00	98.34	0.053
6/17/19	N	7.11	0.00	2.19	0.00	0.00	0.00	0.00	83.14	-0.121
6/19/19	N	7.68	0.00	2.19	0.00	0.00	0.00	0.00	87.13	*N/A
6/21/19	N	7.25	0.00	1.76	0.00	0.00	0.00	0.00	64.57	0.007
6/24/19	N	6.03	0.00	1.98	0.00	0.00	0.00	0.00	78.33	0.037
6/26/19	N	3.08	0.00	1.92	0.00	0.00	0.00	0.00	73.37	0.020
6/28/19	N	6.24	0.00	2.64	0.00	0.00	0.00	0.00	22.43	0.043
7/1/19	N	6.35	0.00	2.52	0.00	0.00	0.00	0.00	19.40	0.040
7/3/19	Y	157	0.00	2.72	0.00	0.00	0.00	0.00	20.51	0.043
7/5/19	N	230	0.00	2.66	0.00	0.00	0.00	0.00	20.15	-0.003
7/8/19	Y	1023	0.00	2.56	0.00	0.00	0.00	0.00	15.04	-0.003
7/10/19	N	17.6	0.00	2.63	0.00	0.00	0.00	0.00	23.52	0.043
7/12/19	N	20.03	0.00	2.76	0.00	0.00	0.00	0.00	21.25	0.043
7/15/19	N	5.98	0.00	2.79	0.00	0.00	0.00	0.00	18.27	0.045
7/17/19	N	3.94	0.00	2.52	0.00	0.00	0.00	0.00	19.47	0.063
7/19/19	N	6.34	0.00	2.75	0.00	0.00	0.00	0.00	48.72	0.050
7/22/19	N	5.27	0.00	2.84	0.00	0.00	0.00	0.00	72.83	0.050
7/24/19	N	7.71	0.00	2.85	0.00	0.00	0.00	0.00	49.04	0.063
7/26/19	Y	220.4	0.00	0.14	0.00	0.00	0.00	0.00	2.56	0.024
7/29/19	N	29.4	0.00	3.14	0.00	0.00	0.00	0.00	20.49	0.061
7/31/19	Y	10.3	0.00	2.97	0.00	0.00	0.00	0.00	28.84	0.061
8/2/19	Y	385.4	0.00	2.95	0.00	0.00	0.00	0.00	47.86	0.061
8/5/19	Y	296.2	0.00	3.00	0.00	0.00	0.00	0.00	29.54	0.061
8/7/19	N	31	0.00	3.15	0.00	0.00	0.00	0.00	18.04	0.034
8/9/19	N	35.4	0.00	3.03	0.00	0.00	0.00	0.00	29.89	0.034
8/12/19	N	103.21	0.00	3.07	0.00	0.00	0.00	0.00	19.63	0.028
8/14/19	N	126	0.00	3.06	0.00	0.00	0.00	0.00	16.11	0.097
8/16/19	N	81.22	0.00	3.09	0.00	0.00	0.00	0.00	16.81	0.097
8/19/19	N	65.4	0.00	3.08	0.00	0.00	0.00	0.00	19.21	0.097
8/21/19	N	50.2	0.00	4.00	0.00	0.00	0.00	0.00	15.84	0.092
8/23/19	N	80.3	0.00	0.20	0.00	0.00	0.00	0.00	22.62	0.248
*June 19th, spectroscopy data not available due to technical problems										

3.7.2 PCA

PCA using nine variables (air temperature in Fahrenheit, water temperature in Celsius, dissolved oxygen, pH, conductivity, rainfall, turbidity, visual turbidity, sulfate levels by IC) measured across each location each day provided assessment of the most

influential variables in determining differences between locations (Figure 3.25). The first two PCs correspond to 23.75% and 20.95% of the total variation in the dataset. A biplot of PCs 1 and 2 establish markers for each individual entity of information graphically represented as a data point. The individual markers are established as single colored data points associated by which sampling location data was collected at.

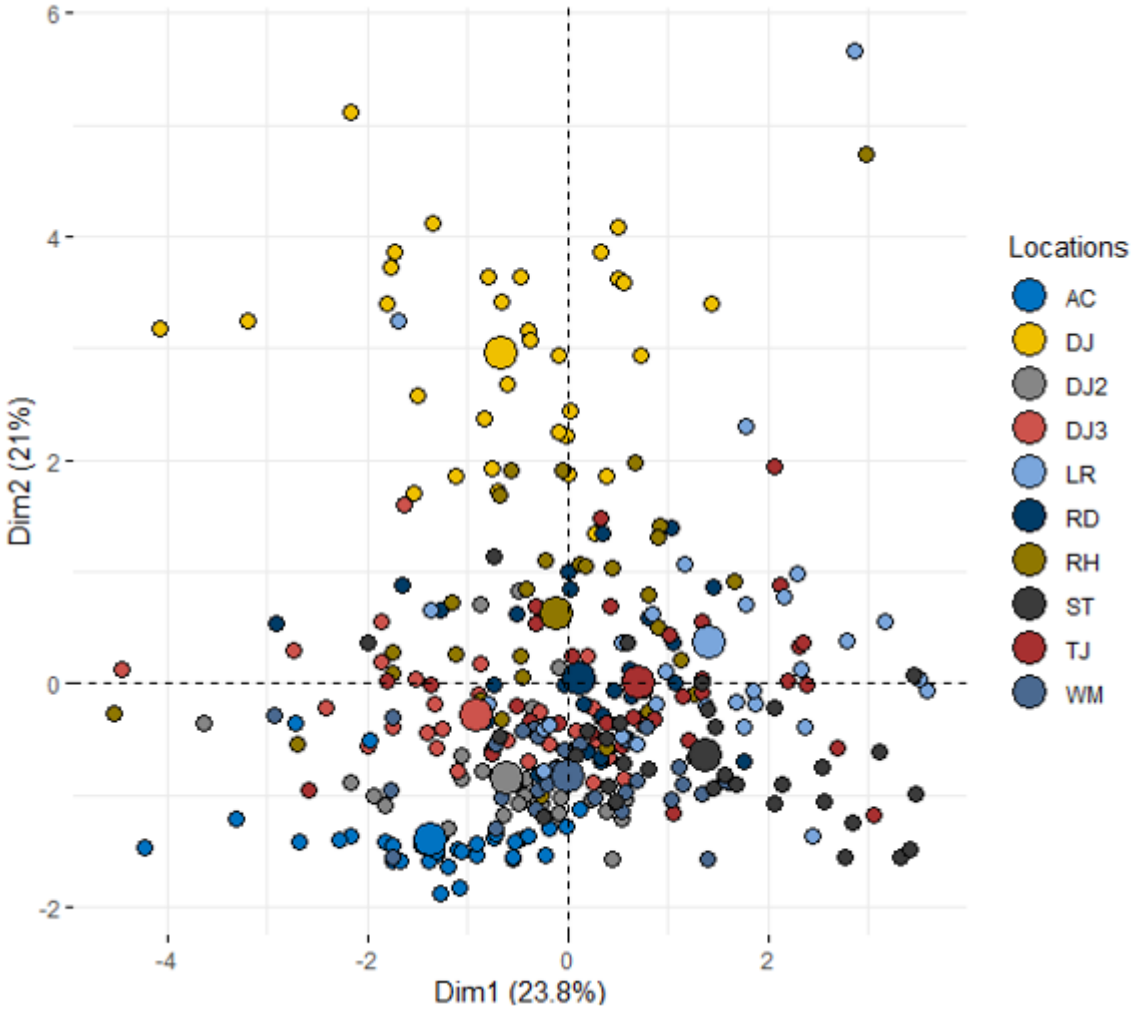

Figure 49 PCA results of individuals marked by location on biplot of PCs 1 and 2.

It was expected to observe high variability percentage established in the first two PCs. This would demonstrate most of the variance between samples could explained with two dimensions, ultimately indicating highly correlative variables. However, this

was not observed with this dataset, indicating that the variables utilized were not highly correlative with each other. Additionally, it was expected that data points would cluster based on either location or by presence of algae. However, data points generally clustered together in the same area near the PC1 axis in the third and fourth quadrant (Figure 3.25).

The only location that was largely separated from other locations was location DJ. To determine the variables that led to this separation, the variable correlation plot is consulted (Figure 3.26). The correlation plot indicates that sulfate presence load positively on PC 2. As already discussed, Location DJ has a natural sulfur spring feeding the water source that leads to higher levels of sulfate present in this location than at others. This is likely the cause of the visual separation between DJ and other locations.

While other locations did not completely separate from each other, Locations LR and RH both had visual outliers, or data points that were plotted separate from the bulk of the location's points. Outliers were evaluated to determine what caused the shift in clustering. Location LR contained three outlier data points and location RH contained 1 data point that each represented individual entity with algal bloom events. Select algal bloom dates were included in this data analysis for this programming so not to overload the software results. The increases in observed conductivity, measured turbidity, and visual turbidity measured during these bloom events drive these data points to be more positively loaded on both PCA1 and PC2 than other data points for Location LR. Although measured turbidity and visual turbidity are indicative of algae specifically, conductivity is an indicative of a change in environment. As trends in these variables

were already observed to roughly correlate with the occurrence of bloom events, this result is not surprisingly. However, the statistical confirmation of the correlation is a welcome conclusion.

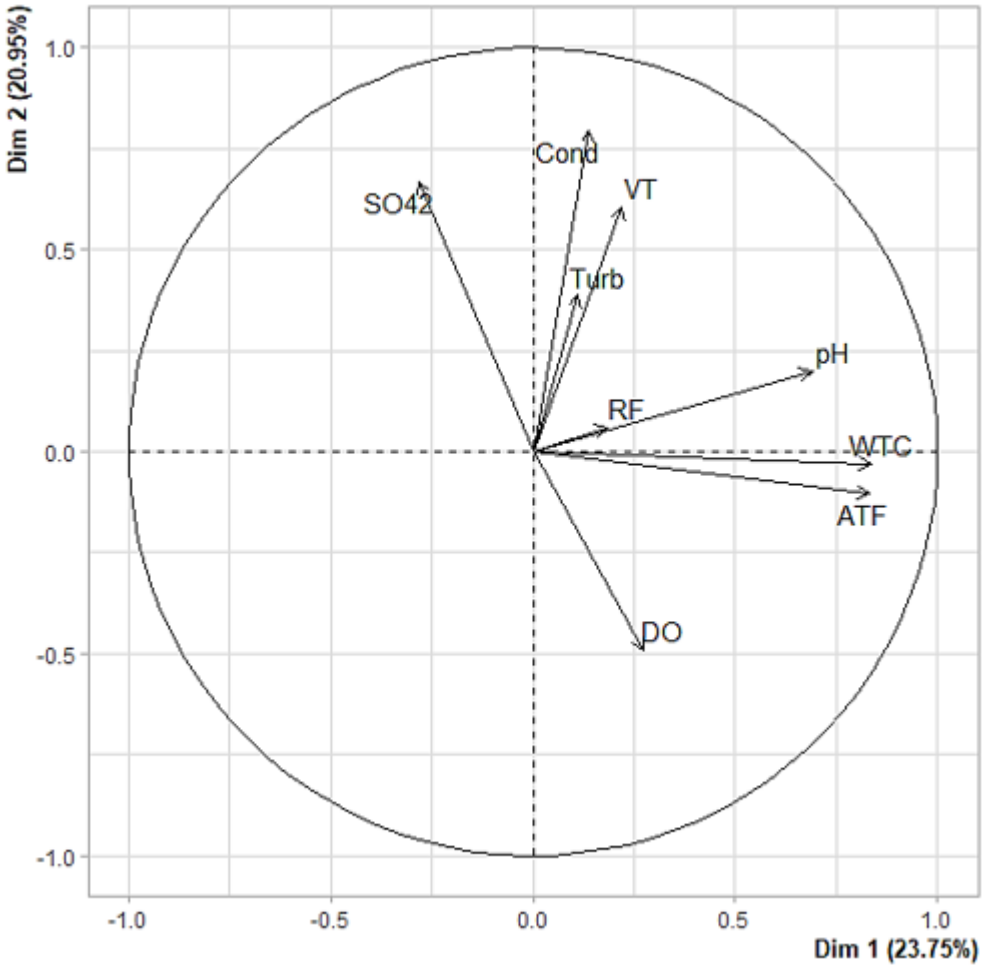

Figure 50 PCA results of variables marked by arrows on monoplot of PCs 1 and 2. Variables within PCA include air temperature in Fahrenheit (ATF), water temperature in Celsius (WTC), pH (pH), dissolved oxygen (DO), rainfall (RF), turbidity (Turb), visual turbidity (VT), conductivity (Cond), and sulfate levels from IC (SO42).

As this technique can be visualized using both plots, PCA is conducted on individual observation dates using a variety of variables. Using the position of the monoplot variables results with the biplot individual data points, outliers were

established conforming to specific variables. Datapoints representing algal bloom events clustered further away from the larger clusters for the location. Outliers were compared with confirmed algal bloom dates to determine which variables led to position changes on the monoplot. Directional arrows were drawn to represent the variable importance to PCA, whereas the datapoints on the biplot fall within relation said variable direction. Algal bloom data points where found in the top quadrants near the sulfate, conductivity, and turbidity variables. PCA established evidence that supports the idea of bloom formation not relying on a set of specific parameters required for growth. This is contradictory to some of what the literature states as the components of algal bloom formation include correlations between temperature, turbidity, and dissolved oxygen. The data supports that there are no underlying specific factors that indicate imminent predisposition for algal bloom formation.

3.7.3 HCA

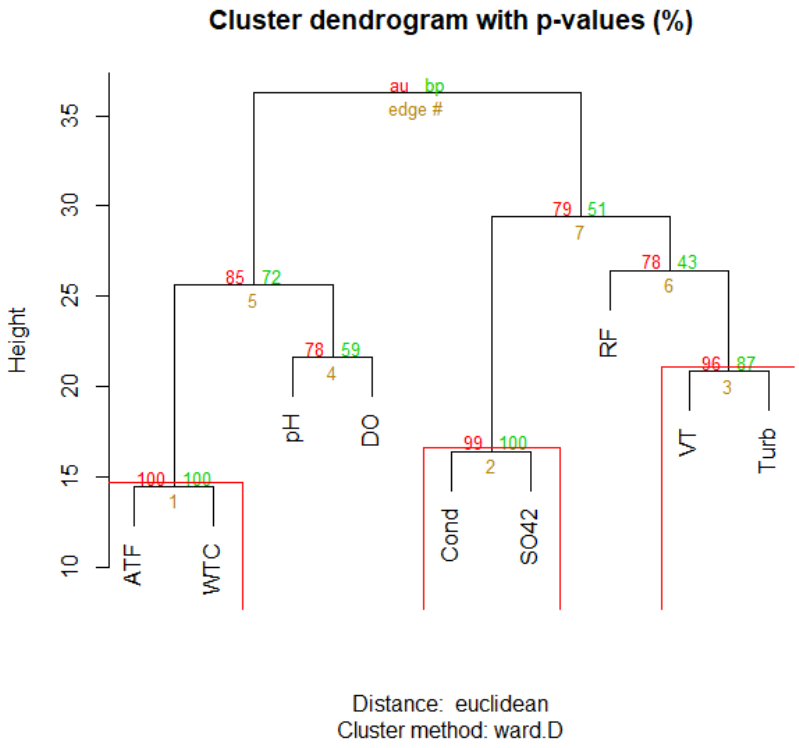

Figure 51 Hierarchical clustering using ward.D method to evaluate variables. Variables within HCA include air temperature in Fahrenheit (ATF), water temperature in Celsius (WTC), pH (pH), dissolved oxygen (DO), rainfall (RF), turbidity (Turb), visual turbidity (VT), conductivity (Cond), and sulfate levels from IC (SO42).

To determine which characteristics, if any, were most strongly correlated to each other, hierarchical clustering was performed. It was expected that air temperature (ATF) and water temperature (WTC) were strongly correlated due to seasonal warming of the air is directly responsible for the warming of water. Additionally, visual turbidity (VT) and quantitative turbidity (Turb) were expected to strongly correlate as they are measuring the same characteristics by two different methods. Rainfall (RF) is somewhat correlative with turbidity. This is due to the sediments being stirred by introduction of water and runoff into the water source. It was expected that pH and dissolved oxygen

(DO) were correlated due to the nature of their direct relationship. As pH values decrease, dissolved oxygen measurements decrease. This is due to the influx of hydrogen ions in the water source. With low pH, less hydrogen ions are within the water source resulting in low dissolved oxygen values. Conductivity (Cond) and sulfate levels (SO_4^{2-}) were expected to have strong correlation due to influx of sulfate species being directly responsible for higher conductivity measurements. Thus, the hierarchical clustering to determine correlations between variables followed expected trends with no additional trends emerging.

Conclusions and Future Work

4.1 Conclusions

This project set out to evaluate local water sources in the same manner as previously studied larger bodies of waters while expecting similar outputs in data. Using identified trends in environmental conditions and nutrient levels, guidelines were expected to be established to help laypersons be better equipped analyzing their private water sources for prediction of algal blooms using commercially available testing kits. While the methods employed in this work were appropriate to attain the expected outcomes, these goals were not easily accomplished. It was determined that understanding harmful algal bloom development is more complex than simply identifying variation in environmental conditions and nutrient levels in locations that may have experienced algal blooms.

While local water sources were evaluated using EPA guidelines and recommended methods to determine nutrient levels, these methods did not support the level of sensitivity needed to quantitate the small levels of nutrients present in these water sources. As the required minimum levels of nutrients for algal bloom development is not clearly defined, it was expected to see concentrations within the instrument's ability of detection when using standard guidelines. Seeing as how this methodology is used in state and federal labs to analyze water samples in larger water sources, it is determined that the necessary amount of nitrates and phosphates required for algal blooms is below quantifiable limits for these local water sources. It was also established that nutrient levels are not the only indicator to be considered for bloom development when evaluating water sources. Though most literature states

eutrophication from high levels of nutrients in required for bloom development, the results of this project contradict this statement and indicate that prediction is more complex.

Environmental conditions were shown to play a role in developing harmful algal blooms in that, if required conditions are not fully met there will be no occurrence of a bloom. The current literature that states that low dissolved oxygen measurements, high turbidity, and increased nutrient levels are required conditions serves as vague guidelines. However, clarified guidelines may not be able to be established as a universal guideline applied to all water sources. This project focused on local water sources not containing large amounts of agricultural runoff and still had occurrences of harmful algal blooms. Although this project did not observe the expected results, it did clarify the parameters for bloom development do not follow strict guidelines that apply to all water sources. Bloom prediction parameters seem to be specific to the water source and surrounding influences. Further study of additional variables should be carried out to fully understand the biological interactions that occur alongside of chemical changes within the water sources.

4.2 Future Work

Continued work for this project would include: conducting growth studies to determine minimum nutrient levels for controlled setting of HAB growth while comparing with in field algae blooms to clarify parameters required for growth, determining new methods to quantitate the minimum required levels of nutrients needed for bloom development, examining the toxins associated with harmful algal blooms

using LC/MS, and continued study into new variables within environmental conditions that could be playing roles in development that weren't studied in this project.

Minimum growth requirements would be evaluated using growth chamber studies for specifically harmful algal species within a lab setting. This would be determined using more sensitive instrumentation to better evaluate the concentrated nutrient levels. Using instrumentation such as ICP-OES, IC-UV, or suppressed IC via conductivity detector, to quantify total phosphorous or total nitrogen present in water samples could be introduced as well. ICP-OES offers lower sensitivity than either the IC or spectrophotometry method used in this project; however, this elemental analysis method does not quantify what form the element is in limiting information about bioavailability and has limited elements that can be analyzed. This applies to total phosphorous but couldn't be used to assess total nitrogen. Using different IC methods, with different detectors or suppressed IC, could achieve lower sensitivity than the IC or spectrophotometry method though. Using these methods, the quantitation of phosphorous and nitrogen could be separated and analyzed at ppb levels. An additional method for analysis could include more sensitive nitrate analysis such as using total Kjeldahl nitrogen to determine the role nitrogen plays in bloom development. Improving this project with similar, yet more specific goals could include 24-hour monitoring of environmental conditions to determine the extent of daily fluctuations or monitoring additional variables such as chlorophyll levels. Increased chlorophyll levels can be monitored using sensor technology or visualization at the water source which indicates bloom development.

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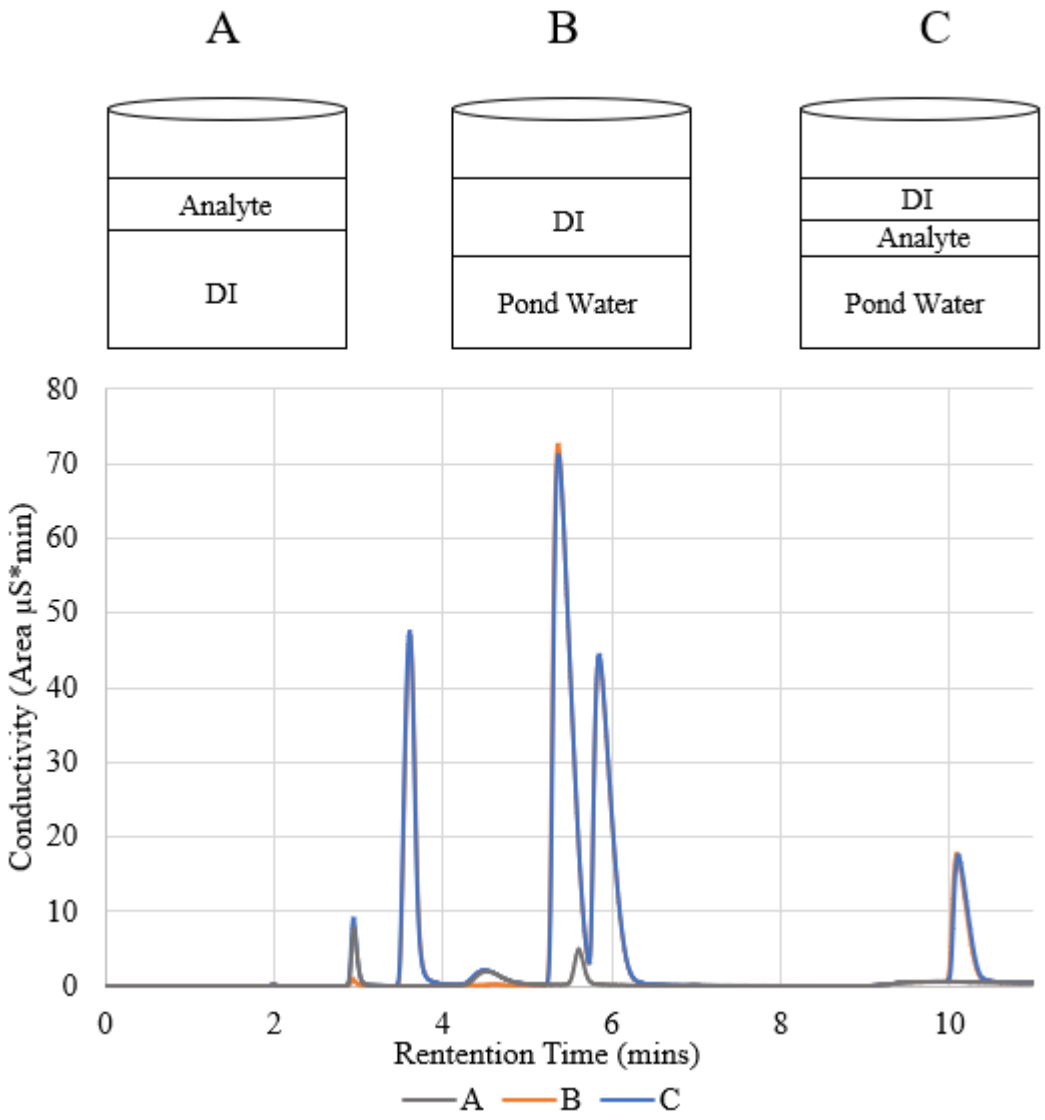
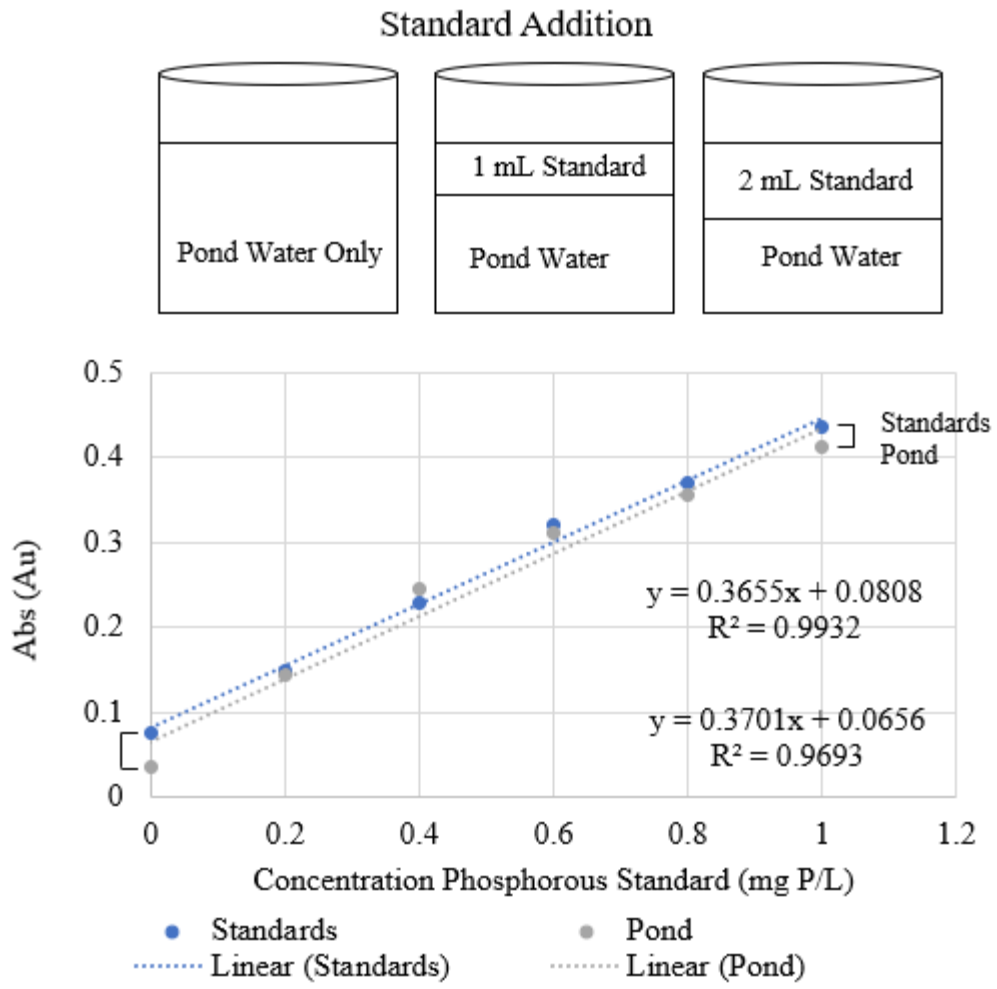
APPENDIX

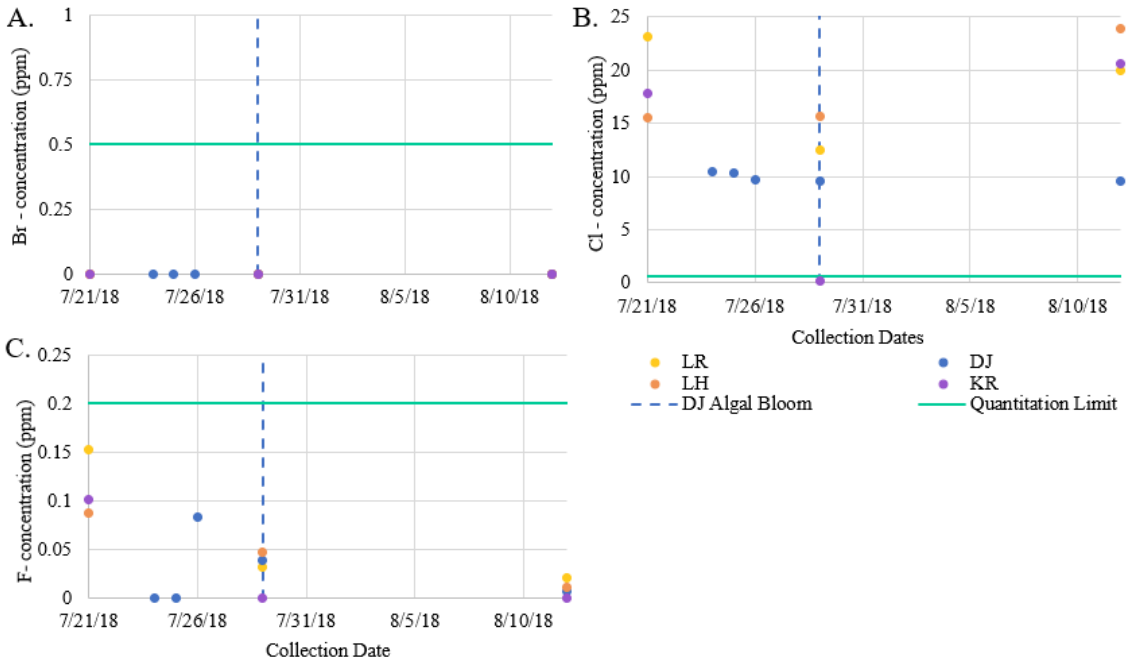
Appendix



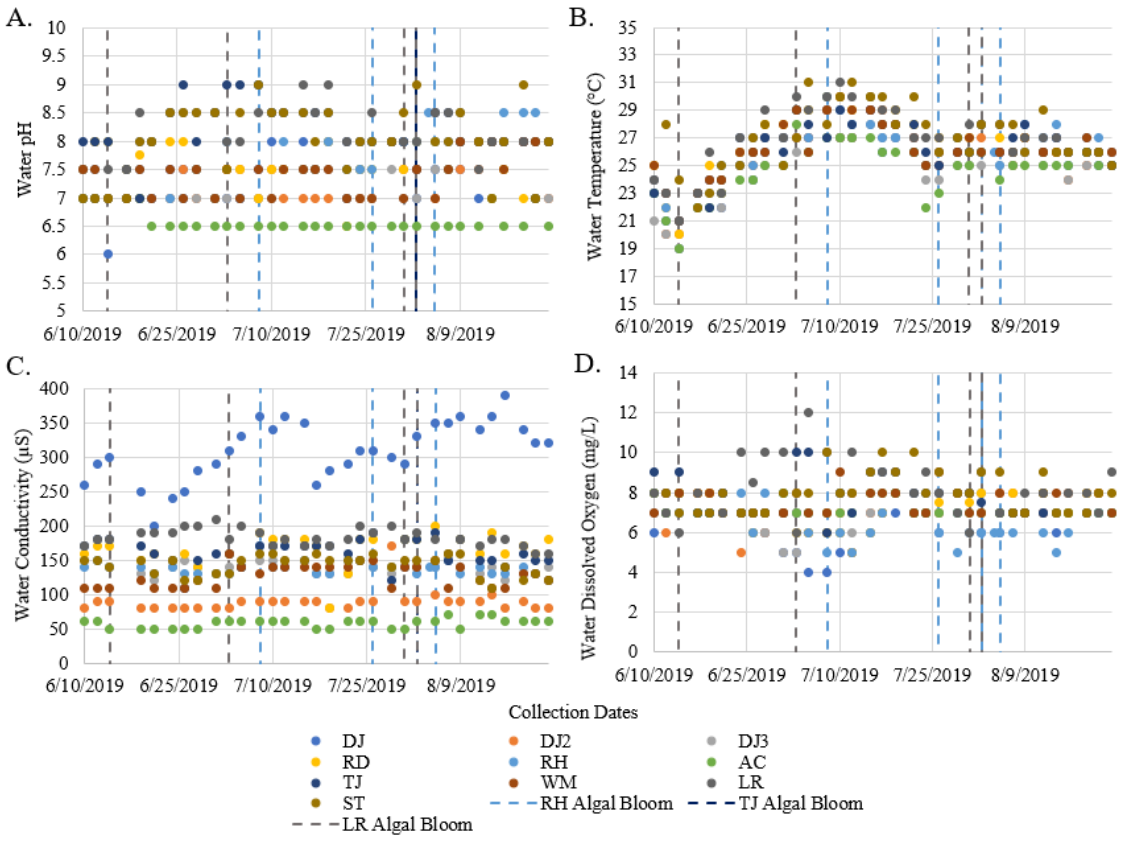
A.1. Matrix interference check with IC 2019



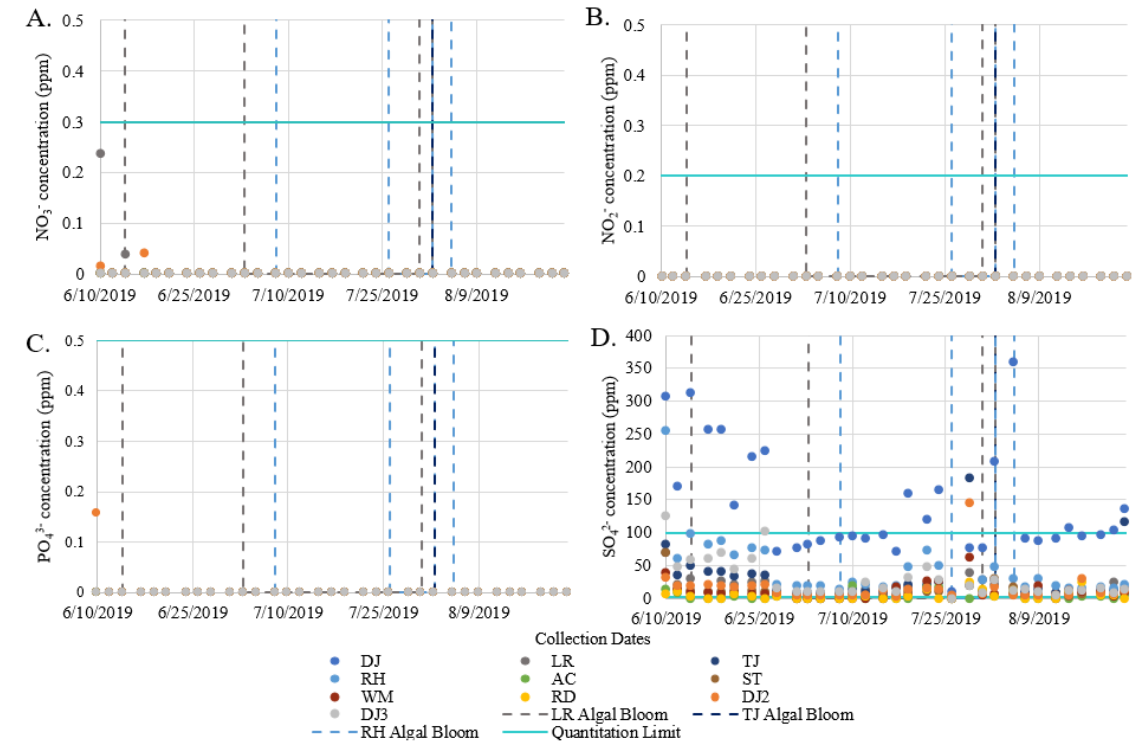
A.2. Matrix interference check with Spectrophotometry 2019



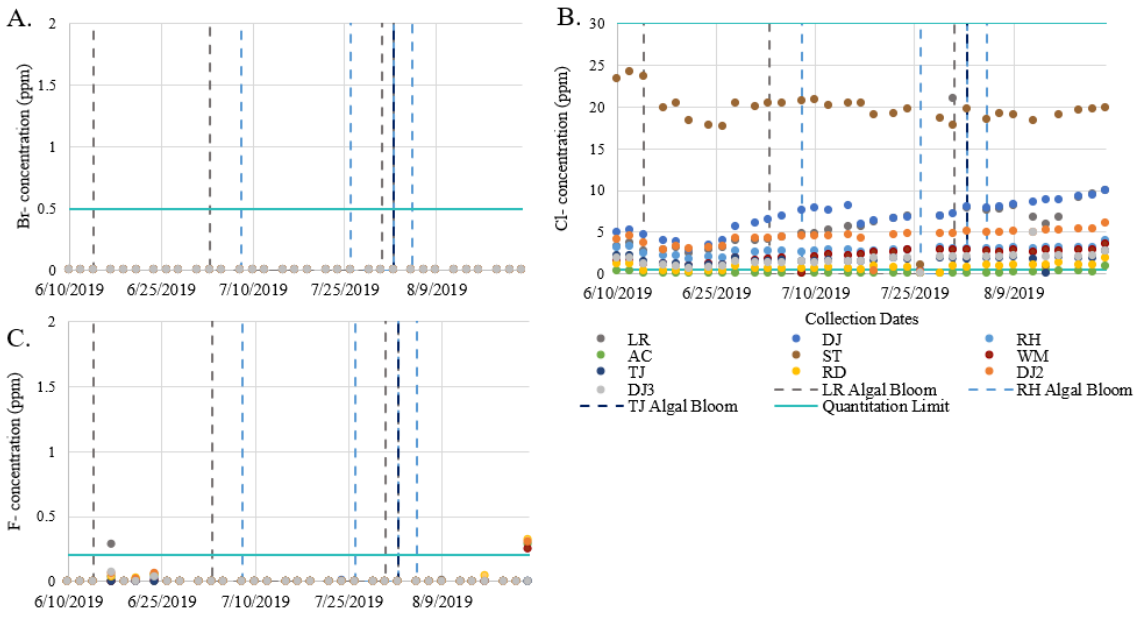
A.3. Ion Chromatography 2018 anion data including A) Bromide, B) Chloride, and C) Fluoride.



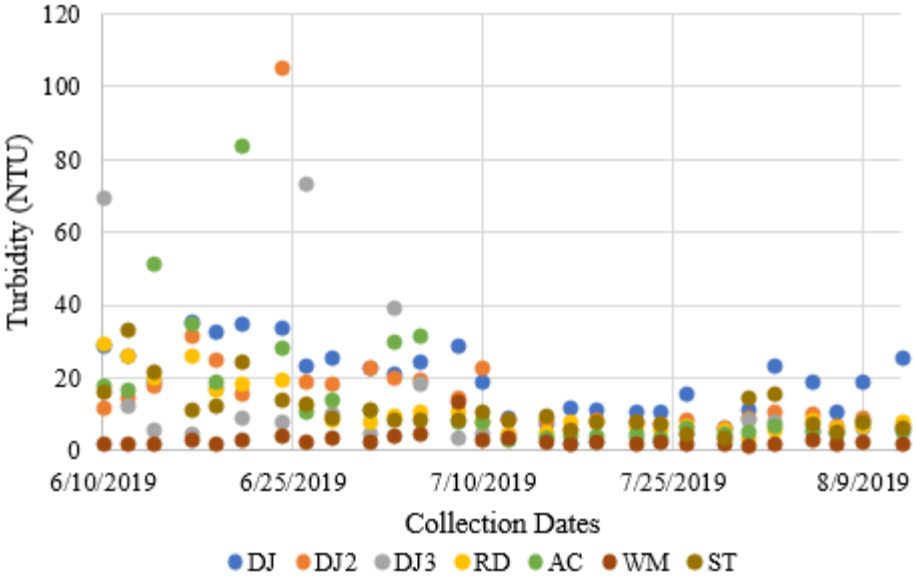
A.4. Summer 2019 environmental condition graphs with all locations including A) pH, B) Water Temperature, C) Conductivity, and D) Dissolved Oxygen.



A.5. 2019 Ion chromatography data for anions of interest of all locations including A) Nitrate, B) Nitrite, C) Orthophosphate, and D) Sulfate.



A.6. 2019 2019 Ion chromatography data for other anions of all locations including A) Bromide, B) Chloride, and C) Fluoride.



A.7. 2019 Turbidity data for locations not affected by algal bloom events.

A.8. Summation data tables for 2019 locations

Sample Site	Doug Jackson Property
Sample ID	"DJ"
City Location	Waco
Latitude	37.765968°
Longitude	-84.109075°
Water source type	Private, farm reservoir
Influences	Agricultural (livestock only), Natural sulfur spring on property

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	72	7.5	23	260	6	1	8:30	9:00	1
6/12/19	N	58	7	21	290	7	2	7:35	7:50	0.25
6/14/19	N	69	6	19	300	8	1	7:41	7:48	0
6/17/19	N	69	7	22	**N/A	7	2	7:49	7:55	0.4
6/19/19	N	70	8	24	250	8	2	7:40	7:48	0.3
6/21/19	N	70	7	22	200	8	2	7:44	7:52	0.45
6/24/19	N	71	7.5	26	240	7	2	7:31	7:37	0.5
6/26/19	N	66	7.5	24	250	6	2	7:31	7:39	0
6/28/19	N	68	7.5	26	280	6	1	7:29	7:35	0
7/1/19	N	68	7	27	290	5	3	7:32	7:39	0
7/3/19	N	71	7	27	310	5	2	7:50	8:03	0.2
7/5/19	N	69	7	27	330	4	2	7:35	7:45	0
7/8/19	N	72	7.5	29	360	4	2	7:31	7:39	0.35
7/10/19	N	73	8	27	340	5	2	7:33	7:39	0
7/12/19	N	69	7.5	27	360	5	2	7:42	7:52	2
7/15/19	N	73	8	29	350	6	2	7:45	7:53	0.8
7/17/19	N	74	7.5	26	260	7	2	7:47	7:55	0.25
7/19/19	N	75	8	27	280	7	2	7:42	7:50	0
7/22/19	N	71	7.5	27	290	7	1	7:47	7:56	0.5
7/24/19	N	58	7.5	25	310	7	2	7:42	7:50	0
7/26/19	N	60	8	25	310	6	2	7:40	7:49	0
7/29/19	N	67	8	26	300	7	2	7:30	7:40	0
7/31/19	N	67	8	26	290	8	1	7:47	7:53	1.2
8/2/19	N	67	7	26	330	6	2	7:46	7:54	0
8/5/19	N	63	7.5	26	350	6	1	7:38	7:45	0
8/7/19	N	69	8	26	350	7	1	7:35	7:41	0
8/9/19	N	69	7.5	26	360	7	2	7:48	7:56	0
8/12/19	N	69	7	26	340	7	1	7:35	7:42	0
8/14/19	N	71	8	27	360	6	2	7:40	7:47	0.3
8/16/19	N	65	8	25	390	7	1	7:45	7:57	0.1
8/19/19	N	68	8	26	340	7	1	7:36	7:45	0.2
8/21/19	N	69	7	25	320	7	1	7:39	7:47	0
8/23/19	N	58	8	25	320	7	1	7:31	7:36	0.2
**June 17th, conductivity measurement not taken due to unavailable instrumentation.										

Sample Site	Doug Jackson Property
Sample ID	"DJ"
City Location	Waco
Latitude	37.765968°
Longitude	-84.109075°
Water source type	Private, farm reservoir
Influences	Agricultural (livestock only), Natural sulfur spring on property

		Turbidity	**Ion Chromatography**							**Spectroscopy**
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	28.7	0.00	4.87	0.00	0.00	0.00	0.00	307.55	0.283
6/12/19	N	25.9	0.00	5.18	0.00	0.00	0.00	0.00	170.39	0.142
6/14/19	N	18.8	0.00	4.64	0.00	0.00	0.00	0.00	314.51	0.074
6/17/19	N	35.4	0.00	3.95	0.03	0.00	0.00	0.00	256.52	0.165
6/19/19	N	32.5	0.00	3.86	0.00	0.00	0.00	0.00	257.74	*N/A
6/21/19	N	34.6	0.00	2.77	0.00	0.00	0.00	0.00	141.82	0.251
6/24/19	N	33.4	0.00	3.46	0.03	0.00	0.00	0.00	215.27	0.151
6/26/19	N	23.5	0.00	3.88	0.00	0.00	0.00	0.00	225.77	0.116
6/28/19	N	25	0.00	5.60	0.00	0.00	0.00	0.00	71.85	0.166
7/1/19	N	22.5	0.00	6.08	0.00	0.00	0.00	0.00	77.64	0.112
7/3/19	N	21.1	0.00	6.46	0.00	0.00	0.00	0.00	83.13	0.104
7/5/19	N	24.1	0.00	6.92	0.00	0.00	0.00	0.00	86.52	0.035
7/8/19	N	28.8	0.00	7.56	0.00	0.00	0.00	0.00	93.86	0.035
7/10/19	N	18.4	0.00	7.87	0.00	0.00	0.00	0.00	95.91	0.125
7/12/19	N	9.03	0.00	7.62	0.00	0.00	0.00	0.00	90.81	0.125
7/15/19	N	7.05	0.00	8.08	0.00	0.00	0.00	0.00	97.14	0.126
7/17/19	N	11.7	0.00	5.92	0.00	0.00	0.00	0.00	70.94	0.194
7/19/19	N	11.4	0.00	6.29	0.00	0.00	0.00	0.00	159.75	0.236
7/22/19	N	10.5	0.00	6.66	0.00	0.00	0.00	0.00	120.43	0.236
7/24/19	N	10.33	0.00	6.70	0.00	0.00	0.00	0.00	166.72	0.194
7/26/19	N	15.2	0.00	0.35	0.00	0.00	0.00	0.00	9.86	0.168
7/29/19	N	5.98	0.00	6.92	0.00	0.00	0.00	0.00	77.14	0.052
7/31/19	N	11.4	0.00	7.16	0.00	0.00	0.00	0.00	77.22	0.052
8/2/19	N	23.1	0.00	7.97	0.00	0.00	0.00	0.00	208.61	0.052
8/5/19	N	18.52	0.00	7.78	0.00	0.00	0.00	0.00	360.01	0.052
8/7/19	N	10.25	0.00	7.95	0.00	0.00	0.00	0.00	90.69	0.051
8/9/19	N	18.52	0.00	8.24	0.00	0.00	0.00	0.00	87.21	0.051
8/12/19	N	25.04	0.00	8.54	0.00	0.00	0.00	0.00	91.12	0.045
8/14/19	N	11.3	0.00	8.85	0.00	0.00	0.00	0.00	106.73	0.049
8/16/19	N	13.2	0.00	8.81	0.00	0.00	0.00	0.00	94.74	0.049
8/19/19	N	21.9	0.00	9.27	0.00	0.00	0.00	0.00	97.36	0.049
8/21/19	N	7.32	0.00	9.41	0.00	0.00	0.00	0.00	104.07	0.044
8/23/19	N	15.2	0.00	9.91	0.00	0.00	0.00	0.00	136.95	-0.011
*June 19th, spectroscopy data not available due to technical problems.										

Sample Site	Doug Jackson Property
Sample ID	"DJ2"
City Location	Waco
Latitude	37.768800°
Longitude	-84.106714°
Water source type	Private, farm reservoir
Influences	Agricultural (livestock only)

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	72	7.5	24	80	8	1	8:32	9:15	1
6/12/19	N	58	7	20	90	6	1	7:59	8:07	0.25
6/14/19	N	52	7	20	90	8	1	7:58	8:04	0
6/17/19	N	69	7	23	**N/A	8	2	8:05	8:13	0.4
6/19/19	N	70	7	24	80	8	1	7:59	8:05	0.3
6/21/19	N	70	7	23	80	8	1	8:04	8:12	0.45
6/24/19	N	71	7	25	80	5	2	7:47	7:58	0.5
6/26/19	N	66	7.5	25	80	7	1	7:50	8:00	0
6/28/19	N	68	7	27	80	6	1	7:46	7:52	0
7/1/19	N	68	7	27	80	7	1	7:49	7:55	0
7/3/19	N	71	7.5	27	80	7	2	8:10	8:15	0.2
7/5/19	N	69	7.5	27	90	6	2	7:53	7:58	0
7/8/19	N	72	7	29	90	7	1	7:47	7:52	0.35
7/10/19	N	73	7.5	27	90	7	1	7:48	7:56	0
7/12/19	N	69	7	27	90	5	1	7:58	8:08	2
7/15/19	N	73	7	29	90	7	1	7:56	8:03	0.8
7/17/19	N	74	7	29	90	7	1	8:02	8:06	0.25
7/19/19	N	75	7	28	80	8	1	8:01	8:06	0
7/22/19	N	71	7	27	80	7	1	7:56	8:05	0.5
7/24/19	N	58	7	25	90	7	1	7:52	8:00	0
7/26/19	N	60	7	25	90	7	1	7:50	8:00	0
7/29/19	N	67	8	26	170	8	1	7:48	7:55	0
7/31/19	N	67	7.5	26	90	8	1	7:59	8:05	1.2
8/2/19	N	67	7.5	27	90	7	1	8:00	8:07	0
8/5/19	N	63	7	26	100	8	1	7:54	7:59	0
8/7/19	N	69	7.5	25	90	8	1	7:45	7:53	0
8/9/19	N	70	7.5	27	90	7	1	8:02	8:09	0
8/12/19	N	69	7.5	26	90	7	1	7:46	7:53	0
8/14/19	N	71	7	26	100	7	1	7:50	8:01	0.3
8/16/19	N	65	8	24	80	7	1	8:00	8:07	0.1
8/19/19	N	69	8	25	90	8	1	7:48	7:57	0.2
8/21/19	N	70	7	26	80	8	1	7:50	7:59	0
8/23/19	N	58	7	26	80	7	1	7:48	7:55	0.2
**June 17th, conductivity measurement not taken due to unavailable instrumentation.										

Sample Site	Doug Jackson Property
Sample ID	"DJ2"
City Location	Waco
Latitude	37.768800°
Longitude	-84.106714°
Water source type	Private, farm reservoir
Influences	Agricultural (livestock only)

		Turbidity	**Ion Chromatography**							**Spectroscopy**
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	11.8	0.00	4.87	0.00	0.00	0.00	0.00	307.55	0.283
6/12/19	N	14.6	0.00	5.18	0.00	0.00	0.00	0.00	170.39	0.142
6/14/19	N	17.8	0.00	4.64	0.00	0.00	0.00	0.00	314.51	0.074
6/17/19	N	31.1	0.00	3.95	0.03	0.00	0.00	0.00	256.52	0.165
6/19/19	N	24.5	0.00	3.86	0.00	0.00	0.00	0.00	257.74	*N/A
6/21/19	N	15.3	0.00	2.77	0.00	0.00	0.00	0.00	141.82	0.251
6/24/19	N	105	0.00	3.46	0.03	0.00	0.00	0.00	215.27	0.151
6/26/19	N	18.5	0.00	3.88	0.00	0.00	0.00	0.00	225.77	0.116
6/28/19	N	18.1	0.00	5.60	0.00	0.00	0.00	0.00	71.85	0.166
7/1/19	N	22.8	0.00	6.08	0.00	0.00	0.00	0.00	77.64	0.112
7/3/19	N	20.1	0.00	6.46	0.00	0.00	0.00	0.00	83.13	0.104
7/5/19	N	19.2	0.00	6.92	0.00	0.00	0.00	0.00	86.52	0.035
7/8/19	N	14.5	0.00	7.56	0.00	0.00	0.00	0.00	93.86	0.035
7/10/19	N	22.9	0.00	7.87	0.00	0.00	0.00	0.00	95.91	0.125
7/12/19	N	8.23	0.00	7.62	0.00	0.00	0.00	0.00	90.81	0.125
7/15/19	N	8.59	0.00	8.08	0.00	0.00	0.00	0.00	97.14	0.126
7/17/19	N	5.68	0.00	5.92	0.00	0.00	0.00	0.00	70.94	0.194
7/19/19	N	8.61	0.00	6.29	0.00	0.00	0.00	0.00	159.75	0.236
7/22/19	N	7.52	0.00	6.66	0.00	0.00	0.00	0.00	120.43	0.236
7/24/19	N	6.97	0.00	6.70	0.00	0.00	0.00	0.00	166.72	0.194
7/26/19	N	8.51	0.00	0.35	0.00	0.00	0.00	0.00	9.86	0.168
7/29/19	N	6.12	0.00	6.92	0.00	0.00	0.00	0.00	77.14	0.052
7/31/19	N	9.03	0.00	7.16	0.00	0.00	0.00	0.00	77.22	0.052
8/2/19	N	10.23	0.00	7.97	0.00	0.00	0.00	0.00	208.61	0.052
8/5/19	N	9.81	0.00	7.78	0.00	0.00	0.00	0.00	360.01	0.052
8/7/19	N	6.57	0.00	7.95	0.00	0.00	0.00	0.00	90.69	0.051
8/9/19	N	8.97	0.00	8.24	0.00	0.00	0.00	0.00	87.21	0.051
8/12/19	N	6.55	0.00	8.54	0.00	0.00	0.00	0.00	91.12	0.045
8/14/19	N	7.21	0.00	8.85	0.00	0.00	0.00	0.00	106.73	0.049
8/16/19	N	8.65	0.00	8.81	0.00	0.00	0.00	0.00	94.74	0.049
8/19/19	N	16.4	0.00	9.27	0.00	0.00	0.00	0.00	97.36	0.049
8/21/19	N	5.18	0.00	9.41	0.00	0.00	0.00	0.00	104.07	0.044
8/23/19	N	8.24	0.00	9.91	0.00	0.00	0.00	0.00	136.95	-0.011
*June 19th, spectroscopy data not available due to technical problems.										

Sample Site	Doug Jackson Property
Sample ID	"DJ3"
City Location	Waco
Latitude	37.767883°
Longitude	-84.105337°
Water source type	Private, farm reservoir
Influences	Agricultural (livestock only)

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	72	7	21	150	7	2	9:24	9:35	1
6/12/19	N	58	8	20	150	7	1	8:13	8:28	0.25
6/14/19	N	52	7	19	140	7	1	8:10	8:18	0
6/17/19	N	70	7.5	22	**N/A	7	1	8:24	8:30	0.4
6/19/19	N	70	7	23	130	8	1	8:11	8:19	0.3
6/21/19	N	70	7	22	120	7	1	8:16	8:22	0.45
6/24/19	N	71	7	25	140	7	1	8:01	8:07	0.5
6/26/19	N	66	7	24	150	7	1	8:05	8:13	0
6/28/19	N	68	7	25	140	6	1	7:55	8:05	0
7/1/19	N	68	7	25	130	5	1	8:01	8:07	0
7/3/19	N	71	7	26	140	5	1	8:20	8:35	0.2
7/5/19	N	69	7	26	140	7	1	8:02	8:07	0
7/8/19	N	72	7	29	150	6	1	8:00	8:10	0.35
7/10/19	N	73	7	27	150	6	1	8:00	8:06	0
7/12/19	N	69	7.5	27	150	6	1	8:11	8:20	2
7/15/19	N	73	7.5	28	150	7	1	8:08	8:15	0.8
7/17/19	N	74	7.5	27	130	7	1	8:10	8:16	0.25
7/19/19	N	75	7.5	27	130	8	1	8:09	8:16	0
7/22/19	N	71	7	26	130	8	1	8:09	8:15	0.5
7/24/19	N	58	7	24	150	7	1	8:02	8:08	0
7/26/19	N	60	7	24	140	7	2	8:04	8:09	0
7/29/19	N	67	7.5	25	130	8	1	7:59	8:05	0
7/31/19	N	67	7	25	140	8	1	8:08	8:13	1.2
8/2/19	N	67	7	25	140	8	1	8:10	8:16	0
8/5/19	N	63	7.5	25	140	7	1	8:03	8:09	0
8/7/19	N	69	7.5	25	150	7	1	7:52	8:01	0
8/9/19	N	70	8	26	140	7	1	8:10	8:16	0
8/12/19	N	70	8	25	130	7	1	8:01	8:07	0
8/14/19	N	71	8	26	140	7	1	8:02	8:07	0.3
8/16/19	N	65	7.5	24	120	7	1	8:09	8:15	0.1
8/19/19	N	69	8	25	130	8	1	8:00	8:07	0.2
8/21/19	N	70	8	26	130	8	1	8:00	8:06	0
8/23/19	N	58	7	25	140	7	1	8:02	8:10	0.2
**June 17th, conductivity measurement not taken due to unavailable instrumentation.										

Sample Site	Doug Jackson Property
Sample ID	"DJ3"
City Location	Waco
Latitude	37.767883°
Longitude	-84.105337°
Water source type	Private, farm reservoir
Influences	Agricultural (livestock only)

		Turbidity	**Ion Chromatography**							**Spectroscopy**
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	69.1	0.00	1.85	0.00	0.00	0.00	0.00	127.49	0.153
6/12/19	N	12.2	0.00	1.88	0.00	0.00	0.00	0.00	48.23	0.048
6/14/19	N	5.52	0.00	1.13	0.00	0.00	0.00	0.00	59.21	-0.011
6/17/19	N	4.25	0.00	0.95	0.06	0.00	0.00	0.00	60.93	-0.141
6/19/19	N	17.3	0.00	0.90	0.00	0.00	0.00	0.00	69.39	*N/A
6/21/19	N	8.98	0.00	0.60	0.00	0.00	0.00	0.00	44.80	0.029
6/24/19	N	7.45	0.00	0.77	0.03	0.00	0.00	0.00	62.10	0.070
6/26/19	N	73.2	0.00	0.88	0.00	0.00	0.00	0.00	102.92	0.089
6/28/19	N	10	0.00	1.40	0.00	0.00	0.00	0.00	15.73	0.077
7/1/19	N	4.39	0.00	1.25	0.00	0.00	0.00	0.00	10.85	0.041
7/3/19	N	39.2	0.00	1.35	0.00	0.00	0.00	0.00	10.92	0.144
7/5/19	N	18.2	0.00	1.37	0.00	0.00	0.00	0.00	10.35	-0.011
7/8/19	N	3.29	0.00	1.40	0.00	0.00	0.00	0.00	9.96	-0.011
7/10/19	N	4.01	0.00	1.37	0.00	0.00	0.00	0.00	9.77	0.043
7/12/19	N	4.27	0.00	1.49	0.00	0.00	0.00	0.00	24.81	0.043
7/15/19	N	3.72	0.00	1.50	0.00	0.00	0.00	0.00	16.75	0.043
7/17/19	N	1.88	0.00	1.42	0.00	0.00	0.00	0.00	10.95	0.049
7/19/19	N	2.41	0.00	1.79	0.00	0.00	0.00	0.00	31.69	0.029
7/22/19	N	3.21	0.00	1.80	0.00	0.00	0.00	0.00	48.38	0.029
7/24/19	N	2.23	0.00	1.90	0.00	0.00	0.00	0.00	28.14	0.049
7/26/19	N	4.2	0.00	0.09	0.00	0.00	0.00	0.00	1.42	0.015
7/29/19	N	3.45	0.00	1.97	0.00	0.00	0.00	0.00	19.29	0.039
7/31/19	N	8.42	0.00	1.99	0.00	0.00	0.00	0.00	8.08	0.039
8/2/19	N	7.43	0.00	1.96	0.00	0.00	0.00	0.00	27.57	0.039
8/5/19	N	5.42	0.00	2.00	0.00	0.00	0.00	0.00	13.04	0.039
8/7/19	N	5.37	0.00	1.98	0.00	0.00	0.00	0.00	0.00	-0.015
8/9/19	N	6.08	0.00	2.09	0.00	0.00	0.00	0.00	9.91	-0.015
8/12/19	N	5.23	0.00	4.89	0.00	0.00	0.00	0.00	4.62	-0.021
8/14/19	N	2.5	0.00	2.07	0.00	0.00	0.00	0.00	0.00	-0.042
8/16/19	N	8.03	0.00	2.12	0.00	0.00	0.00	0.00	13.04	-0.042
8/19/19	N	6.14	0.00	2.05	0.00	0.00	0.00	0.00	15.43	-0.042
8/21/19	N	4.32	0.00	2.07	0.00	0.00	0.00	0.00	8.68	-0.047
8/23/19	N	6.31	0.00	2.73	0.00	0.00	0.00	0.00	0.00	-0.021
*June 19th, spectroscopy data not available due to technical problems.										

Sample Site	Audrey Cooper Property
Sample ID	"AC"
City Location	Waco
Latitude	37.765458°
Longitude	-84.112521°
Water source type	Private reservoir
Influences	Agricultural (surrounded by agribusiness properties)

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	72	7	23	60	8	1	10:27	10:40	1
6/12/19	N	60	7	21	60	7	2	9:10	9:19	0.25
6/14/19	N	51	7	19	50	8	1	8:52	8:56	0
6/17/19	N	72	7	22	**N/A	7	2	9:00	9:10	0.4
6/19/19	N	69	7	22	50	7	1	7:40	7:46	0.3
6/21/19	N	65	6.5	23	50	7	2	8:42	8:52	0.45
6/24/19	N	72	6.5	24	50	7	1	8:39	8:48	0.5
6/26/19	N	69	6.5	24	50	7	1	8:50	9:00	0
6/28/19	N	70	6.5	25	50	7	1	8:35	8:49	0
7/1/19	N	69	6.5	25	60	8	1	8:35	8:45	0
7/3/19	N	73	6.5	28	60	7	1	9:10	9:15	0.2
7/5/19	N	70	6.5	26	60	7	1	8:41	8:46	0
7/8/19	N	72	6.5	27	60	7	1	8:57	9:02	0.35
7/10/19	N	74	6.5	27	60	7	1	8:31	8:36	0
7/12/19	N	69	6.5	27	60	7	1	8:35	8:46	2
7/15/19	N	73	6.5	27	60	8	2	8:52	9:00	0.8
7/17/19	N	76	6.5	26	50	8	2	8:41	8:46	0.25
7/19/19	N	75	6.5	26	50	8	2	8:37	8:45	0
7/22/19	N	73	6.5	26	60	7	1	8:39	8:45	0.5
7/24/19	N	59	6.5	22	60	7	1	8:40	8:48	0
7/26/19	N	62	6.5	23	60	7	1	8:37	8:47	0
7/29/19	N	67	6.5	25	50	7	1	7:05	7:15	0
7/31/19	N	68	6.5	25	50	7	1	8:38	8:44	1.2
8/2/19	N	68	6.5	26	60	7	1	8:45	8:51	0
8/5/19	N	65	6.5	24	60	7	1	8:38	8:43	0
8/7/19	N	69	6.5	25	70	7	1	8:27	8:33	0
8/9/19	N	72	6.5	25	50	7	1	8:37	8:44	0
8/12/19	N	71	6.5	25	70	8	1	8:40	8:46	0
8/14/19	N	72	7	25	70	7	1	8:32	8:40	0.3
8/16/19	N	66	6.5	25	60	7	1	8:42	8:50	0.1
8/19/19	N	70	6.5	26	60	7	1	8:50	8:57	0.2
8/21/19	N	71	7	25	60	7	1	8:49	8:55	0
8/23/19	N	60	6.5	25	60	7	1	8:41	8:49	0.2
**June 17th, conductivity measurement not taken due to unavailable instrumentation.										

Sample Site	Audrey Cooper Property
Sample ID	"AC"
City Location	Waco
Latitude	37.765458°
Longitude	-84.112521°
Water source type	Private reservoir
Influences	Agricultural (surrounded by agribusiness properties)

		Turbidity	**Ion Chromatography**							**Spectroscopy**
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	17.7	0.00	0.27	0.00	0.00	0.00	0.00	13.42	0.068
6/12/19	N	16.1	0.00	0.28	0.00	0.00	0.00	0.00	8.87	0.044
6/14/19	N	51.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.041
6/17/19	N	34.4	0.00	0.00	0.00	0.00	0.00	0.00	0.43	-0.034
6/19/19	N	18.6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*N/A
6/21/19	N	83.7	0.00	0.00	0.01	0.00	0.00	0.00	3.23	0.030
6/24/19	N	27.9	0.00	0.00	0.04	0.00	0.00	0.00	1.19	0.048
6/26/19	N	10.7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.032
6/28/19	N	14.2	0.00	0.00	0.00	0.00	0.00	0.00	2.48	0.052
7/1/19	N	11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.031
7/3/19	N	29.6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.053
7/5/19	N	31.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.101
7/8/19	N	7.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.101
7/10/19	N	7.43	0.00	0.00	0.00	0.00	0.00	0.00	19.73	0.083
7/12/19	N	2.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.083
7/15/19	N	3.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.084
7/17/19	N	3.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.047
7/19/19	N	3.68	0.00	0.00	0.00	0.00	0.00	0.00	0.86	0.026
7/22/19	N	4.2	0.00	0.00	0.00	0.00	0.00	0.00	17.45	0.026
7/24/19	N	3.26	0.00	0.00	0.00	0.00	0.00	0.00	18.13	0.047
7/26/19	N	6.21	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.019
7/29/19	N	4.14	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.036
7/31/19	N	5.23	0.00	0.00	0.00	0.00	0.00	0.00	13.26	0.036
8/2/19	N	6.41	0.00	0.00	0.00	0.00	0.00	0.00	1.66	0.036
8/5/19	N	5.05	0.00	0.07	0.00	0.00	0.00	0.00	11.20	0.036
8/7/19	N	4.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.054
8/9/19	N	7.27	0.00	0.16	0.00	0.00	0.00	0.00	1.15	0.054
8/12/19	N	5.42	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.048
8/14/19	N	2.16	0.00	0.30	0.00	0.00	0.00	0.00	0.00	-0.001
8/16/19	N	5.08	0.00	0.30	0.00	0.00	0.00	0.00	2.73	-0.001
8/19/19	N	6.54	0.00	0.30	0.00	0.00	0.00	0.00	2.37	-0.001
8/21/19	N	4.99	0.00	0.17	0.00	0.00	0.00	0.00	1.35	-0.006
8/23/19	N	5.02	0.00	0.91	0.28	0.00	0.00	0.00	0.00	0.011
*June 19th, spectroscopy data not available due to technical problems.										

Sample Site	Rodney Dixon Property
Sample ID	"RD"
City Location	Waco
Latitude	37.770039°
Longitude	-84.102297°
Water source type	Private, farm reservoir
Influences	Agricultural (Agribusiness, Livestock, Hay)

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	74	7.5	25	160	8	2	9:40	9:55	1
6/12/19	N	59	8	22	170	7	2	8:31	8:41	0.25
6/14/19	N	50	8	20	170	8	2	8:23	8:29	0
6/17/19	N	69	7.5	23	**N/A	8	2	8:39	8:42	0.4
6/19/19	N	70	7.75	25	150	8	1	8:20	8:30	0.3
6/21/19	N	71	8	24	160	7	3	8:57	9:05	0.45
6/24/19	N	71	8	26	150	7	1	8:15	8:21	0.5
6/26/19	N	68	8	26	160	6	2	8:37	8:42	0
6/28/19	N	68	7.5	28	140	7	1	8:15	8:19	0
7/1/19	N	68	7	28	130	7	1	8:10	8:15	0
7/3/19	N	72	7.5	29	160	6	1	8:37	8:46	0.2
7/5/19	N	70	7.5	29	140	6	1	8:16	8:21	0
7/8/19	N	72	7	30	190	6	1	8:20	8:25	0.35
7/10/19	N	74	7.5	30	180	6	1	8:45	8:50	0
7/12/19	N	70	7.5	29	180	5	2	8:53	9:00	2
7/15/19	N	73	7.5	30	180	6	2	8:26	8:32	0.8
7/17/19	N	74	7.5	28	170	7	2	8:22	8:27	0.25
7/19/19	N	75	8	29	80	8	2	8:19	8:25	0
7/22/19	N	71	6.5	28	130	7	2	8:16	8:25	0.5
7/24/19	N	59	7.5	25	180	7	2	8:13	8:20	0
7/26/19	N	60	7.5	27	180	7.5	1	8:10	8:15	0
7/29/19	N	67	8	27	140	8	1	8:06	8:11	0
7/31/19	N	67	7.5	27	180	7.5	1	8:17	8:25	1.2
8/2/19	N	67	8	28	180	8	1	8:20	8:26	0
8/5/19	N	63	8	27	200	7	2	8:15	8:22	0
8/7/19	N	69	7.5	26	160	8	1	8:04	8:10	0
8/9/19	N	71	8	27	180	7	2	8:18	8:23	0
8/12/19	N	70	8	26	160	8	1	8:10	8:16	0
8/14/19	N	72	8	26	190	7	1	8:09	8:14	0.3
8/16/19	N	65	7.5	26	160	7	2	8:19	8:25	0.1
8/19/19	N	71	7	26	170	8	1	8:15	8:24	0.2
8/21/19	N	70	8	26	160	8	1	8:14	8:22	0
8/23/19	N	60	8	26	180	7	1	8:16	8:24	0.2

**June 17th, conductivity measurement not taken due to unavailable instrumentation.

Sample Site	Rodney Dixon Property
Sample ID	"RD"
City Location	Waco
Latitude	37.770039°
Longitude	-84.102297°
Water source type	Private, farm reservoir
Influences	Agricultural (Agribusiness, Livestock, Hay)

		Turbidity	**Ion Chromatography**							**Spectroscopy**
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	29.5	0.00	1.19	0.00	0.00	0.00	0.00	5.45	0.146
6/12/19	N	25.8	0.00	1.16	0.00	0.00	0.00	0.00	9.85	0.048
6/14/19	N	19.4	0.00	0.36	0.00	0.00	0.00	0.00	2.11	0.009
6/17/19	N	25.6	0.00	0.34	0.03	0.00	0.00	0.00	0.95	-0.097
6/19/19	N	16.2	0.00	0.35	0.00	0.00	0.00	0.00	0.93	*N/A
6/21/19	N	18	0.00	0.16	0.02	0.00	0.00	0.00	5.79	0.061
6/24/19	N	18.9	0.00	0.34	0.05	0.00	0.00	0.00	3.09	0.029
6/26/19	N	11.9	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.052
6/28/19	N	8.42	0.00	0.83	0.00	0.00	0.00	0.00	3.24	0.036
7/1/19	N	7.64	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.021
7/3/19	N	9.42	0.00	0.64	0.00	0.00	0.00	0.00	0.00	0.046
7/5/19	N	10.2	0.00	0.67	0.00	0.00	0.00	0.00	0.00	-0.009
7/8/19	N	10.1	0.00	0.66	0.00	0.00	0.00	0.00	0.00	-0.009
7/10/19	N	7.49	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.043
7/12/19	N	4.53	0.00	0.68	0.00	0.00	0.00	0.00	1.59	0.043
7/15/19	N	4.97	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.045
7/17/19	N	7.58	0.00	0.48	0.00	0.00	0.00	0.00	0.00	0.151
7/19/19	N	7.24	0.00	0.83	0.00	0.00	0.00	0.00	2.53	0.174
7/22/19	N	6.42	0.00	0.79	0.00	0.00	0.00	0.00	15.09	0.174
7/24/19	N	5.63	0.00	0.78	0.00	0.00	0.00	0.00	1.38	0.151
7/26/19	N	6.43	0.00	0.03	0.00	0.00	0.00	0.00	0.11	0.111
7/29/19	N	5.62	0.00	0.06	0.00	0.00	0.00	0.00	24.19	0.095
7/31/19	N	3.12	0.00	0.89	0.00	0.00	0.00	0.00	14.07	0.095
8/2/19	N	5.42	0.00	0.79	0.00	0.00	0.00	0.00	2.17	0.095
8/5/19	N	8.31	0.00	0.96	0.00	0.00	0.00	0.00	12.78	0.095
8/7/19	N	6.21	0.00	0.85	0.00	0.00	0.00	0.00	0.00	0.059
8/9/19	N	6.34	0.00	1.03	0.00	0.00	0.00	0.00	0.52	0.059
8/12/19	N	7.35	0.00	0.96	0.00	0.00	0.00	0.00	0.46	0.052
8/14/19	N	9.15	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.037
8/16/19	N	8.63	0.00	1.28	0.04	0.00	0.00	0.00	23.17	0.037
8/19/19	N	5.96	0.00	0.97	0.00	0.00	0.00	0.00	3.63	0.037
8/21/19	N	8	0.00	1.00	0.00	0.00	0.00	0.00	3.71	0.032
8/23/19	N	6.11	0.00	1.80	0.32	0.00	0.00	0.00	0.00	0.007
*June 19th, spectroscopy data not available due to technical problems.										

Sample Site	Tommy Jones Property
Sample ID	"TJ"
City Location	Waco
Latitude	37.786365°
Longitude	-84.133054°
Water source type	Private, farm reservoir
Influences	Agricultural (livestock only)

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	71	8	23	170	9	3	10:50	11:00	1
6/12/19	N	61	8	23	180	8	1	9:25	9:45	0.25
6/14/19	N	56	8	21	180	9	0	9:10	9:19	0
6/17/19	N	73	7.5	23	**N/A	7	1	9:25	9:30	0.4
6/19/19	N	69	7	22	170	7	1	6:35	6:45	0.3
6/21/19	N	66	8	24	160	8	2	9:22	9:28	0.45
6/24/19	N	76	8.5	27	150	7	1	9:01	9:07	0.5
6/26/19	N	74	9	26	110	7	2	9:10	9:15	0
6/28/19	N	74	8	27	150	7	2	9:10	9:15	0
7/1/19	N	72	8.5	26	160	9	2	8:55	9:05	0
7/3/19	N	78	9	29	160	10	1	9:25	9:35	0.2
7/5/19	N	75	9	28	140	10	2	8:56	9:02	0
7/8/19	N	75	9	27	170	6	3	9:16	9:23	0.35
7/10/19	N	76	8.5	29	170	8	2	9:05	9:10	0
7/12/19	N	73	8.5	28	170	7	2	9:11	9:20	2
7/15/19	N	74	8.5	29	170	8	2	9:05	9:12	0.8
7/17/19	N	77	8.5	28	170	9	2	8:58	9:05	0.25
7/19/19	N	76	8.5	28	170	9	2	8:59	9:04	0
7/22/19	N	73	8	28	160	8	1	9:00	9:08	0.5
7/24/19	N	62	8	26	180	8	1	8:55	9:03	0
7/26/19	N	65	8	25	190	8	1	8:53	8:59	0
7/29/19	N	71	8	26	120	8	1	8:20	8:28	0
7/31/19	N	70	8	26	180	8	1	8:52	9:00	1.2
8/2/19	Y	68	7.5	28	180	7.5	2	9:05	9:10	0
8/5/19	N	65	8.5	26	190	7	1	8:56	9:06	0
8/7/19	N	71	8.5	27	150	7	1	8:46	8:51	0
8/9/19	N	75	8	28	180	7	1	8:49	8:55	0
8/12/19	N	71	8	26	150	7	1	8:52	9:00	0
8/14/19	N	72	8	27	150	9	1	8:52	9:05	0.3
8/16/19	N	68	8	26	140	7	1	8:53	9:00	0.1
8/19/19	N	72	8	26	160	8	1	9:00	9:06	0.2
8/21/19	N	73	8	26	150	7	1	9:01	9:08	0
8/23/19	N	61	8	25	150	8	1	8:57	9:04	0.2
**June 17th, conductivity measurement not taken due to unavailable instrumentation.										

Sample Site	Tommy Jones Property
Sample ID	"TJ"
City Location	Waco
Latitude	37.786365°
Longitude	-84.133054°
Water source type	Private, farm reservoir
Influences	Agricultural (livestock only)

		Turbidity	**Ion Chromatography**							**Spectroscopy**
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	9.52	0.00	2.16	0.00	0.00	0.00	0.00	82.79	0.022
6/12/19	N	3.84	0.00	2.18	0.00	0.00	0.00	0.00	35.25	-0.003
6/14/19	N	4.49	0.00	1.37	0.00	0.00	0.00	0.00	49.63	-0.012
6/17/19	N	15.1	0.00	1.18	0.00	0.00	0.00	0.00	41.43	-0.092
6/19/19	N	10.1	0.00	1.21	0.00	0.00	0.00	0.00	42.69	*N/A
6/21/19	N	15.1	0.00	0.84	0.00	0.00	0.00	0.00	33.48	0.040
6/24/19	N	13.3	0.00	1.02	0.00	0.00	0.00	0.00	38.49	0.081
6/26/19	N	20.6	0.00	1.07	0.00	0.00	0.00	0.00	36.20	0.091
6/28/19	N	16.2	0.00	1.80	0.00	0.00	0.00	0.00	12.41	0.088
7/1/19	N	24.7	0.00	1.42	0.00	0.00	0.00	0.00	8.36	0.105
7/3/19	N	26	0.00	1.49	0.00	0.00	0.00	0.00	8.11	0.132
7/5/19	N	20	0.00	1.55	0.00	0.00	0.00	0.00	8.06	0.063
7/8/19	N	15.9	0.00	1.50	0.00	0.00	0.00	0.00	7.79	0.063
7/10/19	N	13.8	0.00	1.46	0.00	0.00	0.00	0.00	15.20	0.077
7/12/19	N	6.02	0.00	1.53	0.00	0.00	0.00	0.00	10.62	0.077
7/15/19	N	6.24	0.00	1.48	0.00	0.00	0.00	0.00	10.01	0.077
7/17/19	N	5.14	0.00	1.39	0.00	0.00	0.00	0.00	16.80	0.025
7/19/19	N	3.08	0.00	1.65	0.00	0.00	0.00	0.00	22.57	-0.005
7/22/19	N	2.5	0.00	1.79	0.00	0.00	0.00	0.00	22.33	-0.005
7/24/19	N	1.59	0.00	1.77	0.00	0.00	0.00	0.00	23.88	0.025
7/26/19	N	5.36	0.00	0.08	0.00	0.00	0.00	0.00	1.92	0.011
7/29/19	N	6.97	0.00	1.82	0.00	0.00	0.00	0.00	183.24	0.028
7/31/19	N	7.89	0.00	1.86	0.00	0.00	0.00	0.00	11.01	0.028
8/2/19	Y	50.8	0.00	1.79	0.00	0.00	0.00	0.00	28.39	0.028
8/5/19	N	15.13	0.00	1.87	0.00	0.00	0.00	0.00	12.07	0.028
8/7/19	N	6.98	0.00	1.79	0.00	0.00	0.00	0.00	0.00	-0.049
8/9/19	N	7.21	0.00	1.96	0.00	0.00	0.00	0.00	13.67	-0.049
8/12/19	N	6.27	0.00	1.77	0.00	0.00	0.00	0.00	0.00	-0.055
8/14/19	N	3.98	0.00	0.10	0.00	0.00	0.00	0.00	0.00	-0.009
8/16/19	N	5.48	0.00	1.93	0.00	0.00	0.00	0.00	12.75	-0.009
8/19/19	N	7.25	0.00	1.90	0.00	0.00	0.00	0.00	13.93	-0.009
8/21/19	N	6.12	0.00	1.93	0.00	0.00	0.00	0.00	11.39	-0.014
8/23/19	N	8.33	0.00	2.75	0.00	0.00	0.00	0.00	0.00	-0.013
*June 19th, spectroscopy data not available due to technical problems.										

Sample Site	William Meade Property
Sample ID	"WM"
City Location	Waco
Latitude	37.787115°
Longitude	-84.131762°
Water source type	Private, recreational reservoir
Influences	Recreational (human activity)

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	71	7.5	25	110	7	1	11:05	11:25	1
6/12/19	N	61	7.5	23	110	8	1	9:50	9:55	0.25
6/14/19	N	56	7	21	110	8	2	9:25	9:30	0
6/17/19	N	73	7	23	**N/A	8	0	9:35	9:40	0.4
6/19/19	N	69	7.5	24	120	8	1	6:49	6:58	0.3
6/21/19	N	66	7	24	110	7	0	9:35	9:40	0.45
6/24/19	N	79	7.5	26	110	7	1	9:11	9:17	0.5
6/26/19	N	74	7	26	110	7	1	9:21	9:30	0
6/28/19	N	72	7.5	26	120	7	1	9:10	9:16	0
7/1/19	N	74	7	28	110	7	1	9:30	9:40	0
7/3/19	N	78	7.5	29	160	8	1	9:45	9:41	0.2
7/5/19	N	75	7	26	140	7	1	9:20	9:25	0
7/8/19	N	75	7.5	29	130	7	1	9:21	9:38	0.35
7/10/19	N	76	7	30	140	9	1	9:20	9:30	0
7/12/19	N	73	7.5	29	140	8	1	9:26	9:33	2
7/15/19	N	74	7.5	29	140	8	1	9:25	9:35	0.8
7/17/19	N	77	7.5	28	140	8	1	9:07	9:13	0.25
7/19/19	N	76	7.5	28	140	8	1	9:06	9:15	0
7/22/19	N	73	7	26	140	7	1	9:12	9:20	0.5
7/24/19	N	62	7	25	150	7	1	9:06	9:15	0
7/26/19	N	65	7	26	150	9	1	9:03	9:08	0
7/29/19	N	71	8	26	110	8	1	8:32	8:40	0
7/31/19	N	70	7	27	140	7	1	9:05	9:11	1.2
8/2/19	N	68	7.5	26	140	7	1	9:15	9:24	0
8/5/19	N	69	7	26	150	8	1	9:10	9:25	0
8/7/19	N	71	7.5	26	110	7	1	8:56	9:05	0
8/9/19	N	75	8	27	140	7	1	9:01	9:09	0
8/12/19	N	72	8	26	120	8	1	9:06	9:17	0
8/14/19	N	72	8	26	110	8	1	9:10	9:16	0.3
8/16/19	N	68	7.5	26	110	8	1	9:03	9:10	0.1
8/19/19	N	72	8	27	130	7	1	9:11	9:18	0.2
8/21/19	N	73	8	26	130	8	1	9:15	9:22	0
8/23/19	N	61	8	26	120	7	1	9:10	9:17	0.2

**June 17th, conductivity measurement not taken due to unavailable instrumentation.

Sample Site	William Meade Property
Sample ID	"WM"
City Location	Waco
Latitude	37.787115°
Longitude	-84.131762°
Water source type	Private, recreational reservoir
Influences	Recreational (human activity)

		Turbidity	**Ion Chromatography**							**Spectroscopy**
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	1.52	0.00	1.52	0.00	0.00	0.00	0.00	40.10	-0.016
6/12/19	N	2.11	0.00	1.78	0.00	0.00	0.00	0.00	13.66	-0.018
6/14/19	N	2.08	0.00	1.20	0.00	0.00	0.00	0.00	8.32	-0.019
6/17/19	N	2.61	0.00	1.10	0.00	0.00	0.00	0.00	7.48	-0.178
6/19/19	N	2.03	0.00	1.06	0.00	0.00	0.00	0.00	6.75	*N/A
6/21/19	N	2.94	0.00	0.88	0.00	0.00	0.00	0.00	8.90	-0.025
6/24/19	N	3.54	0.00	1.12	0.00	0.00	0.00	0.00	7.38	0.003
6/26/19	N	2.5	0.00	1.08	0.00	0.00	0.00	0.00	6.06	0.005
6/28/19	N	3.08	0.00	1.80	0.00	0.00	0.00	0.00	5.31	0.009
7/1/19	N	2.55	0.00	1.65	0.00	0.00	0.00	0.00	0.00	0.008
7/3/19	N	3.87	0.00	1.71	0.00	0.00	0.00	0.00	0.00	0.029
7/5/19	N	4.28	0.00	1.90	0.00	0.00	0.00	0.00	0.00	-0.028
7/8/19	N	13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.028
7/10/19	N	2.95	0.00	2.05	0.00	0.00	0.00	0.00	0.00	0.024
7/12/19	N	3.12	0.00	2.26	0.00	0.00	0.00	0.00	0.04	0.024
7/15/19	N	2.29	0.00	2.14	0.00	0.00	0.00	0.00	0.00	0.024
7/17/19	N	2.08	0.00	2.32	0.00	0.00	0.00	0.00	17.86	0.025
7/19/19	N	2.55	0.00	2.57	0.00	0.00	0.00	0.00	5.12	-0.005
7/22/19	N	1.99	0.00	2.57	0.00	0.00	0.00	0.00	26.93	-0.005
7/24/19	N	2.58	0.00	2.82	0.00	0.00	0.00	0.00	26.47	0.025
7/26/19	N	2.08	0.00	0.14	0.00	0.00	0.00	0.00	1.11	0.001
7/29/19	N	1.54	0.00	2.81	0.00	0.00	0.00	0.00	62.83	0.035
7/31/19	N	0.931	0.00	2.78	0.00	0.00	0.00	0.00	3.80	0.035
8/2/19	N	1.34	0.00	2.89	0.00	0.00	0.00	0.00	7.14	0.035
8/5/19	N	2.65	0.00	2.76	0.00	0.00	0.00	0.00	7.20	0.035
8/7/19	N	1.73	0.00	2.54	0.00	0.00	0.00	0.00	0.00	-0.019
8/9/19	N	2.5	0.00	2.80	0.00	0.00	0.00	0.00	20.45	-0.019
8/12/19	N	1.32	0.00	2.60	0.00	0.00	0.00	0.00	0.00	-0.025
8/14/19	N	1.76	0.00	2.83	0.00	0.00	0.00	0.00	0.00	0.012
8/16/19	N	3.42	0.00	2.72	0.00	0.00	0.00	0.00	9.78	0.012
8/19/19	N	2.01	0.00	2.80	0.00	0.00	0.00	0.00	10.38	0.012
8/21/19	N	2.98	0.00	2.87	0.00	0.00	0.00	0.00	2.34	0.007
8/23/19	N	3.92	0.00	3.50	0.25	0.00	0.00	0.00	0.00	0.053
*June 19th, spectroscopy data not available due to technical problems.										

Sample Site	Lake Reba Property
Sample ID	"LR"
City Location	Richmond
Latitude	37.737179°
Longitude	-84.254860°
Water source type	Public, recreational reservoir
Influences	Recreational (human activity), Agricultural (fertilizer use from surrounding golf courses)

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	74	7	24	170	8	1	11:45	11:52	0
6/12/19	N	68	7	23	180	7	2	10:18	10:22	0
6/14/19	Y	62	7.5	21	180	6	2	9:55	10:15	0.5
6/17/19	N	73	7.5	23	**N/A	8	0	10:05	10:10	0.21
6/19/19	N	70	8.5	26	190	7	1	8:46	8:50	0.2
6/21/19	N	76	8	22	190	10	3	10:04	10:11	0.4
6/24/19	N	79	8.5	27	190	8	1	9:38	9:45	0.3
6/26/19	N	79	8.5	27	200	8.5	2	9:50	10:05	0
6/28/19	N	79	8.5	29	200	9	0	9:45	9:57	0.45
7/1/19	N	76	8.5	27	210	8	1	9:36	9:43	0.25
7/3/19	Y	82	8	30	180	6	1	10:05	10:15	0.5
7/5/19	N	79	8	29	200	7	1	9:31	9:44	0
7/8/19	N	76	8.5	30	190	8	1	10:05	10:15	0
7/10/19	N	81	8.5	31	170	8	1	9:50	9:55	0.1
7/12/19	N	77	8.5	30	180	8	2	9:55	10:03	1.75
7/15/19	N	80	9	30	170	9	2	10:05	10:10	0.1
7/17/19	N	78	8.5	29	180	8	2	9:37	9:45	0.9
7/19/19	N	82	9	29	170	9	2	9:40	9:45	0.25
7/22/19	N	73	8	27	180	8	1	9:30	9:38	1.9
7/24/19	N	68	8	24	200	9	1	9:35	9:42	0
7/26/19	N	71	8.5	27	190	8	2	9:27	9:35	0
7/29/19	N	76	8	27	200	7	2	8:55	9:04	0
7/31/19	Y	75	8	28	180	6	3	9:35	9:42	1
8/2/19	Y	79	8	28	190	6	3	9:45	9:55	0
8/5/19	N	79	8.5	28	180	7	2	9:36	9:50	0
8/7/19	N	74	8.5	26	160	7	1	9:20	9:26	0
8/9/19	N	78	8.5	27	180	8	1	9:28	9:34	0
8/12/19	N	75	7.5	27	170	8	2	9:30	9:36	0
8/14/19	N	77	8	27	180	7	2	9:29	9:36	1.5
8/16/19	N	72	8	26	180	8	1	9:31	9:37	0
8/19/19	N	74	8	26	170	8	1	9:50	9:56	0
8/21/19	N	75	7	26	160	7	1	9:42	9:49	0
8/23/19	N	65	8	25	160	9	1	9:38	9:45	0.1

**June 17th, conductivity measurement not taken due to unavailable instrumentation.

Sample Site	Lake Reba Property
Sample ID	"LR"
City Location	Richmond
Latitude	37.737179°
Longitude	-84.254860°
Water source type	Public, recreational reservoir
Influences	Recreational (human activity), Agricultural (fertilizer use from surrounding golf courses)

		Turbidity	**Ion Chromatography**							**Spectroscopy**
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	15.5	0.00	3.24	0.00	0.00	0.24	0.00	69.63	0.063
6/12/19	N	8.59	0.00	3.64	0.00	0.00	0.00	0.00	22.91	0.016
6/14/19	Y	822	0.00	2.76	0.00	0.00	0.04	0.00	29.99	1.814
6/17/19	N	5.8	0.00	2.82	0.28	0.00	0.00	0.00	54890.31	-0.053
6/19/19	N	27.6	0.00	3.11	0.00	0.00	0.00	0.00	26.45	*N/A
6/21/19	N	13.3	0.00	2.43	0.00	0.00	0.00	0.00	21.96	0.067
6/24/19	N	8.07	0.00	2.81	0.05	0.00	0.00	0.00	24.80	0.039
6/26/19	N	223	0.00	3.18	0.00	0.00	0.00	0.00	25.97	0.338
6/28/19	N	15.2	0.00	3.95	0.00	0.00	0.00	0.00	9.46	0.045
7/1/19	N	3.84	0.00	3.92	0.00	0.00	0.00	0.00	5.43	0.009
7/3/19	Y	13.4	0.00	4.09	0.00	0.00	0.00	0.00	5.18	0.045
7/5/19	N	12.14	0.00	4.40	0.00	0.00	0.00	0.00	5.43	0.286
7/8/19	N	7.61	0.00	4.72	0.00	0.00	0.00	0.00	5.64	0.286
7/10/19	N	9.32	0.00	4.81	0.00	0.00	0.00	0.00	6.84	0.033
7/12/19	N	8.02	0.00	5.23	0.00	0.00	0.00	0.00	15.04	0.033
7/15/19	N	10.42	0.00	5.61	0.00	0.00	0.00	0.00	6.05	0.039
7/17/19	N	6.18	0.00	5.68	0.00	0.00	0.00	0.00	5.89	0.038
7/19/19	N	6.93	0.00	6.20	0.00	0.00	0.00	0.00	18.18	0.013
7/22/19	N	6.84	0.00	6.59	0.00	0.00	0.00	0.00	19.01	0.013
7/24/19	N	5.89	0.00	6.82	0.00	0.00	0.00	0.00	26.48	0.038
7/26/19	N	10.65	0.00	0.35	0.00	0.00	0.00	0.00	1.05	0.038
7/29/19	N	5.14	0.00	6.86	0.00	0.00	0.00	0.00	38.99	0.061
7/31/19	Y	124.3	0.00	21.00	0.00	0.00	0.00	0.00	27.65	0.061
8/2/19	Y	1614	0.00	7.90	0.00	0.00	0.00	0.00	22.28	0.061
8/5/19	N	15.67	0.00	7.63	0.00	0.00	0.00	0.00	16.44	0.061
8/7/19	N	8.49	0.00	7.75	0.00	0.00	0.00	0.00	9.36	-0.041
8/9/19	N	22.07	0.00	8.14	0.00	0.00	0.00	0.00	9.21	-0.041
8/12/19	N	30.85	0.00	6.68	0.00	0.00	0.00	0.00	7.87	-0.047
8/14/19	N	2.12	0.00	5.94	0.00	0.00	0.00	0.00	6.76	-0.022
8/16/19	N	6.91	0.00	6.67	0.00	0.00	0.00	0.00	7.86	-0.022
8/19/19	N	10.73	0.00	9.10	0.00	0.00	0.00	0.00	11.97	-0.022
8/21/19	N	15.42	0.00	9.57	0.00	0.00	0.00	0.00	23.95	-0.027
8/23/19	N	26.9	0.00	9.95	0.00	0.00	0.00	0.00	15.82	0.042
*June 19th, spectroscopy data not available due to technical problems.										

Sample Site	Stratton Pond Property
Sample ID	"ST"
City Location	Richmond
Latitude	37.727848°
Longitude	-84.305738°
Water source type	Public, recreational reservoir
Influences	Recreational (human activity)

Date	Bloom (Y/N)	Air Temperature F°	pH	Water Temperature C°	Conductivity (µS)	DO (mg/L)	Visual Turbidity	Time Start (AM)	Time End (AM)	48 hr. rainfall (in)
6/10/19	N	75	7	28	150	8	2	12:15	12:21	0
6/12/19	N	69	7	24	150	7	3	10:35	10:39	0
6/14/19	N	63	7	22	140	7	2	10:30	10:35	0.5
6/17/19	N	73	7	23	**N/A	7	0	10:15	10:25	0.21
6/19/19	N	70	8	25	150	8	1	9:00	9:05	0.2
6/21/19	N	73	8	25	130	7	1	10:23	10:28	0.4
6/24/19	N	76	8.5	27	150	7	1	10:08	10:15	0.3
6/26/19	N	80	8.5	28	120	7	1.5	10:15	10:30	0
6/28/19	N	80	8.5	25	120	8	1	10:05	10:15	0.45
7/1/19	N	77	8.5	27	130	8	1	9:55	10:05	0.25
7/3/19	N	85	7.5	31	130	8	1	10:25	10:30	0.5
7/5/19	N	82	8.5	28	150	10	1	9:55	10:04	0
7/8/19	N	80	9	30	160	8	1	10:30	10:35	0
7/10/19	N	82	8.5	31	160	8	2	10:05	10:12	0.1
7/12/19	N	81	8.5	30	150	9	1	10:20	10:26	1.75
7/15/19	N	82	8.5	30	150	10	1	10:13	10:18	0.1
7/17/19	N	78	8	28	160	9	1	9:53	9:59	0.9
7/19/19	N	83	8.5	30	150	10	1	9:55	10:05	0.25
7/22/19	N	73	7.5	28	150	8	1	9:50	10:05	1.9
7/24/19	N	70	8	26	150	9	1	9:52	10:00	0
7/26/19	N	75	8	27	160	8	2	9:57	10:04	0
7/29/19	N	76	8	26	140	8	1	9:11	9:19	0
7/31/19	N	75	8.5	28	150	9	1	10:08	10:15	1
8/2/19	N	79	9	28	150	9	1	10:10	10:17	0
8/5/19	N	79	8	28	150	7	1	10:05	10:15	0
8/7/19	N	74	8	26	160	7	1	9:49	9:54	0
8/9/19	N	79	8.5	29	160	9	1	9:40	9:46	0
8/12/19	N	75	8	26	120	7	1	9:45	9:52	0
8/14/19	N	77	7	26	110	7	1	9:45	9:55	1.5
8/16/19	N	73	8	26	140	7	1	9:48	9:54	0
8/19/19	N	76	9	26	120	8	1	10:05	10:12	0
8/21/19	N	75	7	25	130	8	1	10:01	10:07	0
8/23/19	N	65	8	25	120	7	1	9:49	9:58	0.1
**June 17th, conductivity measurement not taken due to unavailable instrumentation.										

Sample Site	Stratton Pond Property
Sample ID	"ST"
City Location	Richmond
Latitude	37.727848°
Longitude	-84.305738°
Water source type	Public, recreational reservoir
Influences	Recreational (human activity)

		Turbidity	**Ion Chromatography**							**Spectroscopy**
Date	**Bloom (Y/N)**	**Turbidity (NTU)**	**Bromide (ppm)**	**Chloride (ppm)**	**Fluoride (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**O-phosphate (ppm)**	**Sulfate (ppm)**	**O-phosphate (ppm)**
6/10/19	N	16	0.00	23.34	0.00	0.00	0.00	0.00	70.42	0.045
6/12/19	N	33.1	0.00	24.09	0.00	0.00	0.00	0.00	15.10	0.073
6/14/19	N	21.3	0.00	23.66	0.00	0.00	0.00	0.00	11.57	-0.018
6/17/19	N	11.3	0.00	19.89	0.00	0.00	0.00	0.00	10.71	-0.126
6/19/19	N	12.3	0.00	20.39	0.00	0.00	0.00	0.00	10.61	*N/A
6/21/19	N	24	0.00	18.23	0.00	0.00	0.00	0.00	12.90	0.182
6/24/19	N	13.7	0.00	17.79	0.00	0.00	0.00	0.00	11.98	0.055
6/26/19	N	12.4	0.00	17.60	0.00	0.00	0.00	0.00	11.32	0.068
6/28/19	N	8.92	0.00	20.37	0.00	0.00	0.00	0.00	5.67	0.061
7/1/19	N	11	0.00	19.94	0.00	0.00	0.00	0.00	1.36	0.035
7/3/19	N	8.51	0.00	20.32	0.00	0.00	0.00	0.00	1.19	0.096
7/5/19	N	8.24	0.00	20.34	0.00	0.00	0.00	0.00	1.43	0.021
7/8/19	N	8.33	0.00	20.64	0.00	0.00	0.00	0.00	1.39	0.021
7/10/19	N	10.3	0.00	20.83	0.00	0.00	0.00	0.00	7.58	0.181
7/12/19	N	8.42	0.00	20.14	0.00	0.00	0.00	0.00	5.11	0.181
7/15/19	N	9.21	0.00	20.43	0.00	0.00	0.00	0.00	2.21	0.176
7/17/19	N	5.62	0.00	20.36	0.00	0.00	0.00	0.00	1.90	0.076
7/19/19	N	8.05	0.00	19.02	0.00	0.00	0.00	0.00	9.75	0.068
7/22/19	N	7.51	0.00	19.11	0.00	0.00	0.00	0.00	10.47	0.068
7/24/19	N	6.82	0.00	19.72	0.00	0.00	0.00	0.00	9.86	0.076
7/26/19	N	4.18	0.00	1.01	0.00	0.00	0.00	0.00	0.61	0.070
7/29/19	N	3.41	0.00	18.52	0.00	0.00	0.00	0.00	20.17	0.053
7/31/19	N	14.5	0.00	17.80	0.00	0.00	0.00	0.00	10.91	0.053
8/2/19	N	15.23	0.00	19.68	0.00	0.00	0.00	0.00	29.68	0.053
8/5/19	N	6.94	0.00	18.49	0.00	0.00	0.00	0.00	18.10	0.053
8/7/19	N	5.12	0.00	19.13	0.00	0.00	0.00	0.00	3.50	-0.024
8/9/19	N	7.63	0.00	18.93	0.00	0.00	0.00	0.00	3.75	-0.024
8/12/19	N	6.24	0.00	18.32	0.00	0.00	0.00	0.00	3.16	-0.030
8/14/19	N	2.78	0.00	0.89	0.00	0.00	0.00	0.00	3.16	-0.004
8/16/19	N	4.99	0.00	18.94	0.00	0.00	0.00	0.00	5.77	-0.004
8/19/19	N	6.23	0.00	19.50	0.00	0.00	0.00	0.00	7.69	-0.004
8/21/19	N	9.05	0.00	19.74	0.00	0.00	0.00	0.00	4.31	-0.010
8/23/19	N	8.1	0.00	19.76	0.00	0.00	0.00	0.00	12.01	-0.017
*June 19th, spectroscopy data not available due to technical problems.										