Summer 2016

# Evaluating the Influences of Karst Hydrogeology on Freshwater Harmful Algal Blooms in Kentucky Lakes

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# EVALUATING THE INFLUENCES OF KARST HYDROGEOLOGY ON FRESHWATER HARMFUL ALGAL BLOOMS IN KENTUCKY LAKES

A Thesis
Presented to
The Faculty of the Department of Geography and Geology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

By
Robert T. Schaefer III

August 2016

EVALUATING THE INFLUENCES OF KARST HYDROGEOLOGY ON FRESHWATER HARMFUL ALGAL BLOOMS IN KENTUCKY LAKES

Date Recommended 20 JUNE 2016

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7/11/16

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## ACKNOWLEDGMENTS

Completing this thesis, and, to a large extent, graduate school in general, to say the least, has been a journey. Journeys are often fraught with heartache and hardship, but they are also full of fun and excitement, and this adventure was no different. I was fortunate enough to be surrounded with a group of amazing friends and a mentor without whom this thesis would not have been possible. It was through this journey that I learned that the completion of a thesis, and graduate school survival, is not a feat completed by an individual alone, but by a group effort propelling an individual to a singular goal.

Friends have always played a large role in helping me push through hardships. If it were not for my two of my closest friends, Ellen Barringer and Zack Sullivan, my sanity would have long ago fled. Thanks to Ellen for lending me an ear when times were most dire and Zack for being by my side throughout this adventure. I would have likely quit long ago without their support. I also need to thank Caleb Koostra and Kegan McClanahan, as they were instrumental in the collection of my research data and, without them, this study would have been very difficult. Helping others with their work helped propel me forward as well. I want to say thank you to Jonathan Oglesby, my fellow artist and friend. Weekend GIS sessions with Joneo kept my skills sharp and our discussions always proved to be a highlight of my week. I want to thank the USACE and, specifically, the Louisville District Biologist, Jennifer Thomason. She helped me navigate the maze of federal policy and research, as well as provided me with much needed data. So many of my friends have helped me with the completion of this document, even if it was not through direct means. Every smile, cheesy joke, and terrible prank kept my spirits high and helped drive me to finish this work. Thank you to all of my friends and colleagues.

My family and loved ones also played a role in the completion of this project. They always supported my efforts from the shadows, even if their only contribution was a simple “you can do this” at the end of a phone call. Thank you all for the never-ending support and well wishing. None of this would have been possible without my mother, Yvonne Woods, my two brothers, Jonathan and Joseph Schaefer, and my best friend, Skylar Bisono. All of my labors were well validated when I came home to those smiling faces and warm hugs.

I also want to thank the people I have come to see as mentors during my time at Western Kentucky University. Dr. Leslie North, your guidance and tutelage in all things education made me the educator I am today. I hope to have a long and great career in this field and I go forward from here with the strong knowledge base you helped me build. Dr. Pat Kambesis, thanks for being “one of the three” and generally being awesome. I revere your knowledge of caving and mapping and, someday, hope to be able to live up to your legend.

Finally, I want to thank my biggest supporter, mentor, guide, thorn-in-my-side, and advisor, Dr. Jason Polk. I have grown so much throughout this process and you have been there to guide me every step of the way. You fought off the demons of paperwork and administration while I toiled in the field, cultivating a thesis. Thank you for helping me become a better writer, scientist, leader, and person. So much of this was only possible because I knew you had my back every step of the way and, for that alone, I will forever consider you my friend.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EVALUATING THE INFLUENCES OF KARST HYDROGEOLOGY ON FRESHWATER HARMFUL ALGAL BLOOMS IN KENTUCKY LAKES

Robert Schaefer August 2016 95 Pages

Directed by: Jason Polk, Leslie North, and Patricia Kambesis

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A problem exists in Nolin River Lake and Rough River Lake in Kentucky, due to the increasing prevalence of cyanobacterial-based harmful algal blooms (CyanoHABs) and the threats they pose to local communities. These lakes were developed as artificial reservoirs from embankment. Further complicating the issue, the lakes are located within a heavily karstified region and there exists no plan or method currently for monitoring or managing CyanoHABs in a karst region with regard to groundwater inputs to the lake systems or their tributaries. A mixture of techniques and analysis methods was used to determine the best way to monitor and possibly detect the formation and occurrence of CyanoHABs in artificial lakes that are located in karst landscapes. The methods focused on determining the effect groundwater has on CyanoHAB occurrence and formation, how much nutrient pollution is entering the system, from where the pollution is originating and, ultimately, how best to monitor and develop management practices against CyanoHAB occurrence. Techniques used included dual nitrate isotope tracing, collecting hydrogeochemical data, lake discharge data, historical CyanoHAB data, and biological tracer monitoring in both lakes. The lakes under study showed varying degrees of the influence karst plays in their seasonal changes from summer to winter pools. Lake water temperatures never dropped below the temperatures needed for one of the dominant cyanobacteria, *Cylindrospermopsis raciborskii*, to grow. Calculations of nutrient loadings indicated that over 3.5 x $10^6$ kg of nitrate moved through Nolin River Lake during the

course of the study. The presence and concentrations of *E. coli* when paired with weather and geochemical data also revealed karst groundwater pulses exerting an influence through the system in response to precipitation events. The nitrogen and oxygen isotope data indicate that a wide variety of nitrate pollution sources are entering the system and that a variety of management techniques must be deployed to combat this complex issue. A holistic approach that focuses on management and education about karst processes and CyanoHABs is suggested, with an emphasis on broader community involvement beyond just the populations living adjacent to the lakes.

# Chapter 1: Introduction

A growing problem exists in Kentucky due to the occurrence of harmful algal blooms (HABs) in lakes and reservoirs. The blooms are forming in several waterways and, particularly, in artificial lakes. HABs do not just consist of green algae, but also contain blue-green algae (cyanobacteria). Cyanobacterial blooms (CyanoHABs) have the potential to produce several life-threating toxins, which pose a threat to humans, wildlife, and the surrounding environment; therefore, local resource managers need methods to better identify the causes of CyanoHABs in order to design best management practices (BMPs) to combat the problem. During the spring and summer of 2013 and 2014, several lakes in Kentucky (formed by dams) were placed under water quality advisories, because of high cell-count densities of cyanobacteria, per World Health Organization (WHO) guidelines (Chorus and Bartram 1999; WHO 2003). Two of the larger affected lakes are Nolin River Lake and Rough River Lake. These lakes play important roles in the local communities as sources of water, recreation, income, and flood control. The United States Army Corps of Engineers (USACE) is the agency responsible for these resources and for managing CyanoHAB occurrences, as these lakes were formed from impoundment dams built along the main rivers from which they are fed.

The currently accepted management practice used to combat CyanoHABs involves limiting nutrient loading in order to reduce the level of eutrophication of the waterway, thus preventing the cyanobacteria from blooming by starvation. This practice is popular because most of the sources of nutrient pollution are anthropogenic and, thus, controllable. Given the variability and lack of data on the drainage basins of Rough River and Nolin River lakes, as is the case for most internally drained and complex karst landscapes, finding and regulating those sources of pollution are often difficult. By testing for the main contributors to eutrophication and the preferred blooming conditions of cyanobacteria, a better understanding was achieved in this study regarding the role karst hydrology plays in delivering the anthropogenic pollution that contributes to the eutrophication of the lakes. Examining the heavy isotopes $^{15}N$ and $^{18}O$ of nitrate ($NO_3$) provides insight on the possible sources of the nitrogen pollution, which often contributes to CyanoHAB occurrences (Aravena and Robertson 1998; Kendall 1998; Fukada et al. 2004; Einsiedl et al. 2005; Kendall et al. 2007; Dähnke et al. 2008; Pellerin et al. 2009; Wankel et al. 2009; Xue et al. 2009; Yuan et al. 2012). This analysis aided in identifying the ancillary sources of nutrient pollution, such as phosphate ($PO_4$). In addition to nutrient pollution, other anthropogenic contaminants, such as *Escherichia coli* (*E. coli*) bacteria, can also be monitored to help further delineate sources of the types of nutrient pollution.

Currently, there does not exist a method or evaluative criteria for determining the hydrogeological influences of karst settings on CyanoHAB occurrences in reservoirs and lakes. The extent is such that CyanoHABs even occur in the winter, under ice, due to the dynamic hydrology from potential groundwater inputs occurring year-round and influencing the water temperature and geochemistry. The primary aim of this study was to develop a method and test for anthropogenic nutrient pollution and contaminants entering the lakes through karst hydrogeologic inputs. Another goal was to develop best management practices (BMPs) to help local resource managers prevent CyanoHAB occurrences. To achieve these goals, the following research questions are the focus of this study:

- What is the best method for identifying and testing karst inputs as an influence on harmful algal blooms in Kentucky lakes?
- What influences do the karst inputs to two reservoirs in central Kentucky have on harmful algal bloom formation?
- What are the concentrations and types of anthropogenic contaminants and nutrient pollution entering these lakes through karst hydrological inputs?
- What are the likely sources of the contaminants and nutrient pollution coming through karst tributaries?

Chapter 2 provides a review of the background research, while these research questions are primarily addressed in Chapter 3, which serves as a manuscript from this research that is being submitted for publication and is written in that format as a stand-alone document. Chapter 4 discusses the broader significance of the research within the context of Kentucky's lakes and beyond.

# Chapter 2: Literature Review

## 2.1 Karst Landscape and Hydrogeology

Karst landscapes cover approximately 15% of the ice-free continental area with 20-25% of the global population depending on karst groundwater for agriculture, drinking water, and recreation (Daoxian and Zaihua 1998; Ford and Williams 2007). Karst landscapes are distinguished by highly interconnected surface and subsurface features (see Figure 2.1), such as sinkholes, sinking streams, springs, blue holes, conduits, and caves (White 1988).

Central and south-central Kentucky's landscape presents unique challenges to resource managers, particularly when concerned with water quality, due to the highly developed karst terrain. Since the mid-1800s, CyanoHABs have formed in U.S. lakes and reservoirs with increasing frequency, with a common progenitor of anthropogenic nutrient pollution (Schindler and Vallentyne 2008; Taranu et al. 2015). In Kentucky, these CyanoHABs are increasingly hard to manage, due to the nature of the karst landscape and unknowns regarding its influence on their occurrence.

Karst features are created by hydrologic interactions between rock and slightly acidic water. Carbonic acid ($H_2CO_3$) forms when naturally occurring carbon dioxide ($CO_2$) dissolves into meteoric waters and falls to the ground as precipitation (Ford and Williams 2007; Palmer 2007). As the water permeates the soil, $CO_2$ concentrations up to two orders of magnitude higher than atmospheric concentrations (405 ppm) further allow $CO_2$ to dissolve into the percolating water creating more carbonic acid (Drever 1982; White and White 1989; Ford and Williams 2007). The carbonic acid solution infiltrates into the fractures of the carbonate bedrock below (Palmer 1991). The most common of these karst-forming rocks is limestone, composed primarily of calcite ($CaCO_3$). The dissociation reaction of calcite into calcium and bicarbonate is shown in the following reaction (Drever 1982; White and White 1989):

$$2H_2O + CO_2 + CaCO_3 \leftrightarrow H_2O + Ca^{2+} + 2HCO_3^- \qquad (2.1)$$

After decades to millennia, the dissolution enlarges networks of conduits to eventually form caves. This allows for more water and acid to enter the system and further dissolve the rock, thereby creating a complex subsurface drainage network.

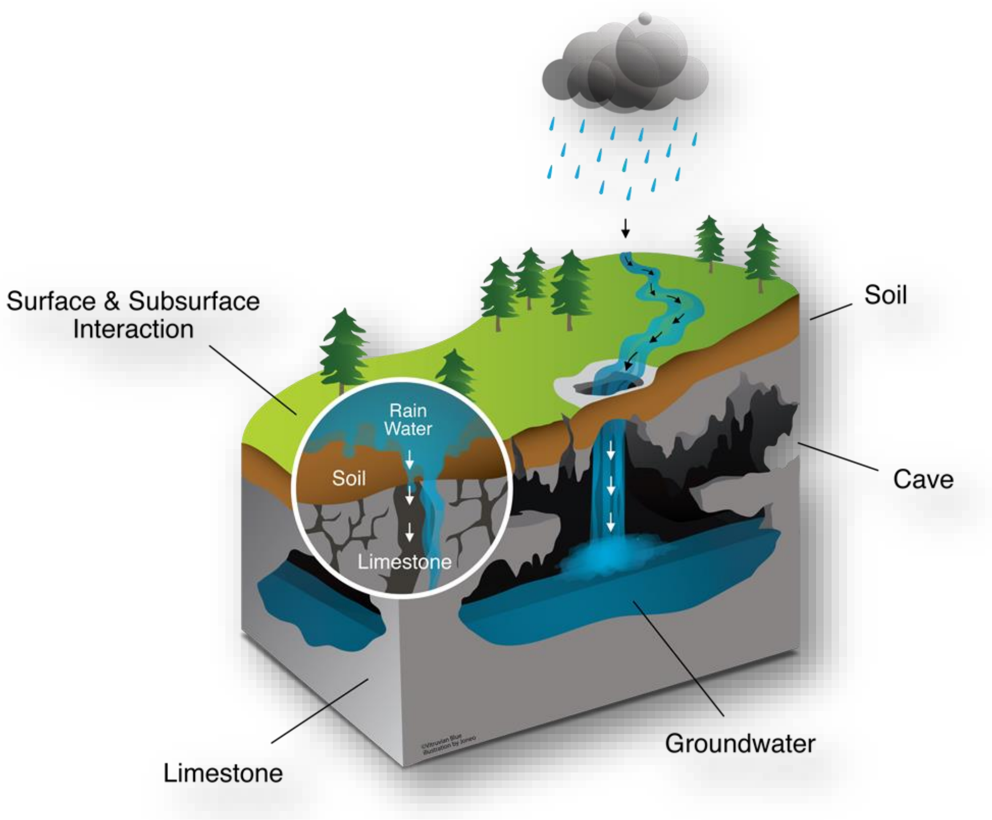



Figure 2.1. Karst landscape diagram. This diagram illustrates the process through which karst landscapes form. Source: Created by Jonathan Oglesby based on UnderBG (2014).

The complex underground network of caves and conduits facilitates the movement of vast quantities of water in relatively short periods of time. Quick movement of water also leads to weathering and erosion processes and allows for the rapid transit of sediments and runoff over distances that would not be possible for surface transit alone (Quinlan and Ewers 1985). These conduits also link the surface waters to groundwater in ways that are not typical in other types of geology. The high level of connectivity allows for rapid transit of contaminants through the aquifer, something often overlooked by water resource managers (White 1988).

Kentucky's aquifers are largely composed of telogenetic karst rock (Hess and White 1988). Telogenetic karst aquifers are characterized by indurated, conduit-dominated bedrock, which allow for more connectivity than other types of karst (Florea and Vacher 2006). The aquifers recharge through discrete surface features and runoff is given a direct path to the groundwater in the aquifers (Ford and Williams 2007). Aquifer water then discharges into the local surface water features such as river, streams, and lakes upon intersecting the water table or at discrete elevations (Marshak 2012). Groundwater often is the source of the water that feeds the local bodies of surface waters in karst regions. With the relatively quick flushing of stormwater runoff, the bacteria, nutrients, and sediments that would normally be filtered out by soil instead get pushed through the watersheds via the groundwater; thus, the amount of time water spends in the system, the residence time, is short (Hess and White 1988). This creates a complex scenario in which runoff rapidly enters the groundwater through surface features and exits through karst outputs with many possible pathways in between, including a complete mixing with other groundwater and a lack of filtration for contaminants.

The traditional way to delineate the sources of karst outputs is chemical dye tracing (Mull 1993). Chemical dye tracing in karst landscapes involves injecting a florescent chemical dye into a groundwater input (i.e., sinkholes, sinking streams, etc.) and then monitoring the outputs of the local area for the dye. This method also allows for researchers to gather useful information on flow paths and flow rates. Multiple dyes may be utilized through different inputs to estimate the level of mixing occurring within the aquifer (Randall and James 2001; Goldscheider and Drew 2007). Isotopic tracing of carbon is also used to determine connections between inputs and outputs (Lee and Krothe 2001). Given the complex nature of a karst groundwater flow system, however, the direct paths contaminants take cannot always be ascertained. This complexity makes the understanding and management of water resources exceedingly difficult in karst areas. Water quality issues, such as eutrophication in lakes and reservoirs, can become compounded by the complex nature of karst groundwater.

## 2.2 Eutrophication of Lakes

Eutrophication is a natural stage in a waterbody's life cycle. As the body of water fills with sediments and becomes shallower, the concentrations of nutrients in the water and sediments increase (Wetzel 2001). This facilitates the growth of more aquatic primary producers, which trap more sediment, slow the rate of flow, and further the processes of succession. This is a process that takes place over the course of several years. Other factors in contributing to eutrophic states in a body of water include high residence times, defined levels of stratification, high turbidity, high specific conductance (SpC), low mixing rates, and minimal dissolved oxygen (DO) (Elçi 2008; Schindler and Vallentyne 2008).

Residence refers to the length of time a particular part of water or sediment takes to leave a body of water. In bodies of water with low flow rates, higher basal temperatures, and high residence time, the water tends to be come stratified. This stratification forms layers with clear differences in temperature, nutrient makeup, and dissolved oxygen content (Elçi 2008). Turbidity is a measure of the opaqueness of water, or how much light can pass through a given water sample. The higher the turbidity, the less light can pass through water. High turbidity also deprives the lower lake depths of solar radiation, which causes thermal stratification (Wetzel 2001). Specific conductance is an important characteristic of water quality and is a measure of the dissolved ion species within the water. It is calculated by the conductance of electricity across a distance of water at a specific temperature. A higher specific conductance means there are more dissolved ions in the water, which is often the case in karst regions. Just a few of these dissolved ions include, but are not limited to, nitrate, sulfate, phosphate, and metal ions (APHA 1992). All of these parameters are of concern to water quality managers and contribute to eutrophication. If the flow in a body of water is low, then the chances of vertical mixing are equally low and stratification of the water column may occur. The vertical mixing helps prevent stratification by moving colder waters to the surface and warmer waters down. The natural process of mixing also helps distribute nutrients and phytoplankton more evenly, aiding in bloom prevention (Rahman 2010); however, in lakes with high turbidity, it can facilitate toxic bloom formation (Zhou et al. 2016). The bottom layers of eutrophic systems tend to be hypolimnal and are more commonly referred to as dead zones (Wetzel 2001). These zones have little or no dissolved oxygen and, as a result, little to no aerobic biological activity. Dissolved oxygen is an indicator of a lake's health, since fish and zooplankton require it for respiration.

Eutrophication can also occur unnaturally in deep water lakes. Deep eutrophic lakes are generalized as lakes that are highly stratified with high levels of biological processes going on near the surface due to an overloading of nutrients (Bonnet et al. 2000). These lakes tend to have poorly defined littoral zones, which trap the nutrients and contaminants in runoff. In a eutrophic system, hypoxia and overcrowding by algae tends to kill off the hydrophytes leaving the littoral zone exposed. Flood control reservoirs suffer a similar problem, but have the added problem of varying depths, moving shores, and underdeveloped littoral zones as a result of summer and winter pools (Lindström 1973). The opposite of eutrophic lakes are oligotrophic lakes, which are characterized by low levels of biological activity and extremely limited nutrient levels. It is possible for a body of water to enter this trophic state after the nutrients in the system are depleted (Wetzel 2001; Schindler and Vallentyne 2008). Monitoring the trophic states of a body of water is important for water quality and safety (Borowski et al. 2012).

Anthropogenic eutrophication is defined as nutrient overloading and contamination from human sources (Schindler 2012). Prior to the science of limnology, nutrient overloading presented itself as a major issue in water resource management and concerns with it continue even today (Vollenweider 1971; Schindler 2006). Examples of anthropogenic sources include agriculture, urban runoff, industrial waste, and human wastewater. Eutrophication occurs globally and in all varieties of water ecosystems (Smith et al. 2006; Borowski et al. 2012). This overloading of nutrients has severe impacts on the local flora and fauna. In many cases, algae and cyanobacteria bloom and choke out the grazers, like zooplankton, which keep those populations under control. The break in the food web has a rippling effect that eventually may lead to the collapse of the ecology of that particular body of water (Wetzel 2001; Paerl 2008; Schindler and Vallentyne 2008). The main contributing nutrients to eutrophication in freshwater systems are inorganic carbon, nitrogen, phosphorus, and other trace elements. Eutrophication of a lake can lead to HAB formation and a plethora of other health and safety issues (Swanson and Baldwin 1965).

**2.3 Contamination through Nutrient Loading**

Carbon was once thought to be the primary limiting nutrient in freshwater systems (Lange 1967; 1971; Goldman et al. 1974) and it is particularly prominent in karst waters; however, it was later shown to play a lesser role than previously thought (Schindler 1971; 1977). The role of carbon in eutrophication is just a step in the carbon cycle, which is a process that occurs on a global scale whereby carbon moves between reservoirs in various stages of inorganic and organic states (Post et al. 1990). Water makes up most of the larger reservoirs of carbon and in eutrophic waters carbon is in such abundance that it is not a limiting factor.

As mentioned previously, $CO_2$ is very soluble in water - almost 200 times more so than oxygen (Wetzel 2001). The solubility of carbon is dependent on many factors, including pH, temperature, and concentration of carbonate ions. At a higher pH (e.g., alkaline environments), $CO_2$ is less likely to dissolve into water, but at lower pH (e.g., acidic environments), $CO_2$ will dominate the system (Golterman and Clymo 1969). In soft water lakes, or lakes with relatively low concentrations of calcium bicarbonate and a relatively neutral pH, the concentrations of dissolved $CO_2$ in atmosphere and water are relatively in equilibrium (Cole et al. 1994). In hard-water lakes, or lakes with high levels of bicarbonate, $CO_2$ will readily dissolve at higher concentrations; this occurs despite higher pH values due to the abundance of hydroxide ions (Wetzel 2001). At a small hard-water lake in Michigan, where the primary tributary was a karst spring, the levels of dissolved $CO_2$ were as much as seven times the higher then atmospheric levels (Otsuki and Wetzel 1974). The $CO_2$ levels were lower in the summer, only three times more than atmospheric concentrations, but still contained more inorganic carbon than soft water systems, since carbonate species tend to dissolve more readily in cold water. In hard-water lakes, an equilibrium forms between bicarbonate, carbonate, calcium, and dissolved calcium carbonate, and $CO_2$. When the concentrations of $CO_2$ are high enough, calcite ($CaCO_3$) precipitates. $CaCO_3$ can encrust the macrophytic vegetation and even encrust them enough that the plants cannot support the weight of the $CaCO_3$ (Wetzel 1960). What makes carbon of concern in reference to eutrophication is its consumption by aquatic plants for photosynthesis. The free-floating algae are not affected by this encrustation of $CaCO_3$ and consume the available $CO_2$. Cyanobacteria even produce carbonates as byproducts of respiration (Wetzel 2001). This lends to furthering the destruction of hydrophytes in the littoral zones. The system forms a positive feedback loop in which the result is detrimental to the freshwater ecosystem's health; however, due to the abundance of inorganic carbon in water, other nutrients tend to serve as important limiting factors in growth and can be managed with more efficiency and a better margin of success.

Although nitrogen is one of the most abundant elements on the planet, making up the majority of the atmosphere by volume, only about 2% of it is available to organisms (Galloway 1998). Nitrogen is very important to life because there is a nitrogenous base in all the nucleic acids that make up deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nitrogen is also one of the key components of adenosine triphosphate (ATP), which the main source of energy for nearly all the cells on Earth.

The nitrogen that organisms use is referred to as reactive nitrogen and is largely created by biological processes. Nitrogen is naturally added to the global ecosystem on the order of 90-130 teragrams per year (90-130 billion kilograms) (Galloway 1998). Humans, however, have greatly disrupted the natural nitrogen cycle (Figure 2.2). A study by Galloway et al. (1995) estimated that an additional 140 teragrams per year (140 billion kilograms) of nitrogen were being added to the global nitrogen cycle from anthropogenic sources, in addition to the naturally occurring nitrogen. These human-introduced sources include fertilizer, fossil fuel combustion, nitrogen-fixing crops, and the mobilization of nitrogen through human disturbance (Vitousek et al. 1997). In the normal nitrogen cycle, non-reactive nitrogen ($N_2$) is transformed into useable, reactive nitrogen-based compounds, such as ammonia ($NH_4^+$), nitrite ($NO_2^-$), and nitrate ($NO_3^-$). This is completed through a few different pathways (Figure 2.2). Some of the compounds enter the system in the form of precipitation and some are pulled out of the atmosphere and solution by *in situ* nitrogen fixation, an ability of some plants, cyanobacteria, and bacteria (Bernhard 2010). Once nitrogen is in the organic system, it remains in the cycle of decay and usage until it leaves again in the form of inactive nitrogen. In water ecosystems, nitrogen plays a large role as a limiting nutrient, because it is not as easily available in a useable form, such as nitrate. Cyanobacteria play an important role in most aquatic systems as a source of reactive nitrogen by affixing nitrogen from the atmosphere using specialized heterocyst cells (Wetzel 2001).

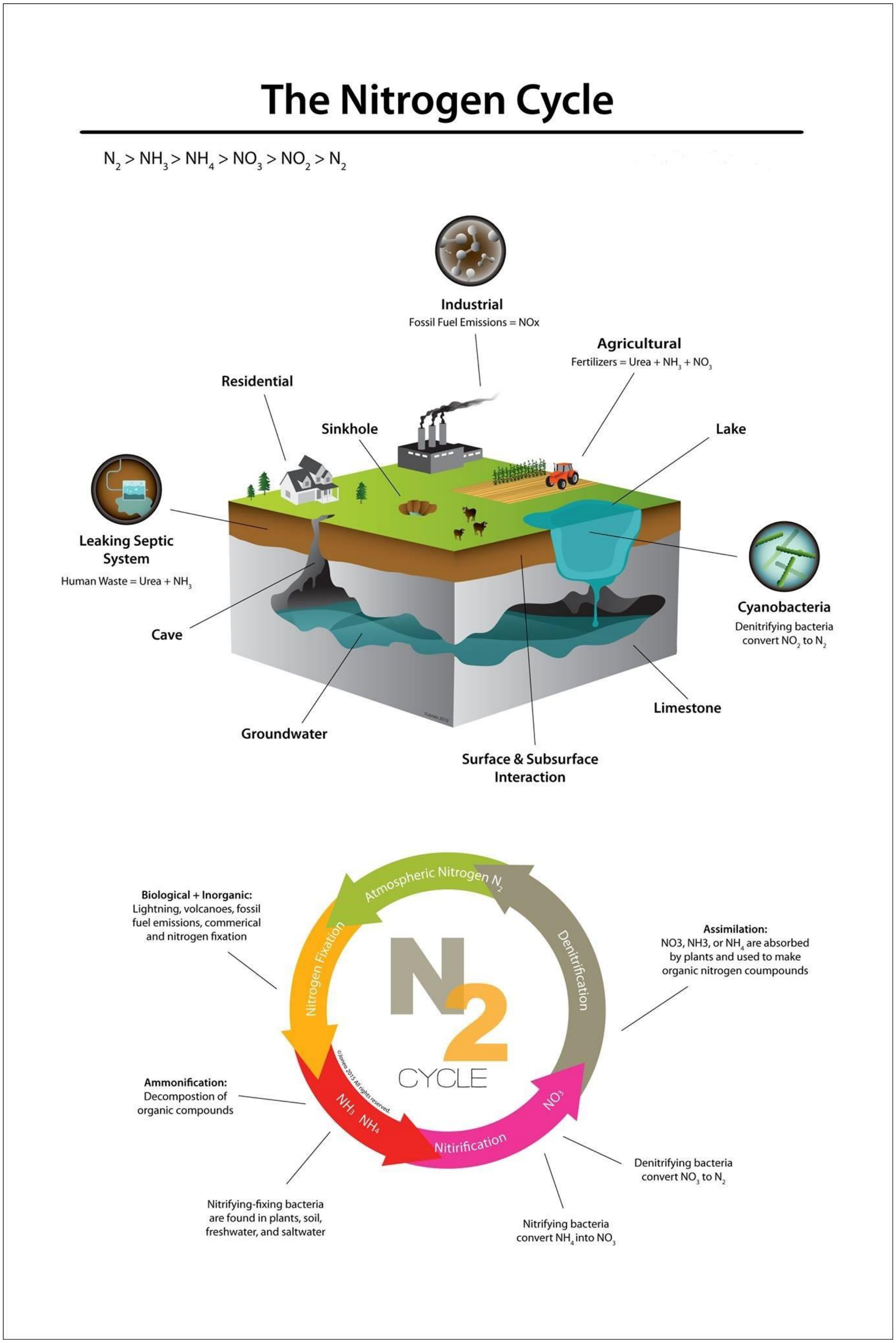



Figure 2.2. The Nitrogen Cycle. The nitrogen cycle is a process in which nitrogen cycles through various organic and inorganic stages before returning to an inactive state. Source: Created by Jonathan Oglesby.

Those systems are mostly in the places where nitrogen is not readily available in the runoff, marine environments, and estuaries. Sources of nitrogen contamination can be traced through the use of stable isotopes (Kellman and Hillaire-Marcel 2003; Katz 2004; Choi et al. 2007; Albertin et al. 2011). Most elements have multiple stable isotopes, or isotopes that do not radioactively decay, under normal conditions. These isotopes only vary from their original element in the number of neutrons present in the nucleus (Clark and Fritz 1997). For nitrogen, there exist two stable isotopes, $^{14}N$ and $^{15}N$. The heavy isotope of nitrate ($\delta^{15}N$-$NO_3$) is generally used to discern these sources. The delta (δ) notation signifies that the following number is a ratio of the isotopic abundance of the sample versus the reference material expressed in a per mil value (‰). The reference material for nitrogen is the $N_2$ found in air. The following is the equation used to calculate $\delta^{15}N$ (Clark and Fritz 1997):

$$\delta^{15}N_{Sample} = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{reference}}{(^{15}N/^{14}N)_{reference}}\ x\ 1000‰\ \text{AIR} \qquad (2.2)$$

By calculating the $\delta^{15}N$ value, the source of the molecule can be discerned. The range of values for $\delta^{15}N$-$NO_3$ in groundwater for different sources are: soil nitrate (-3‰ to +14‰), inorganic fertilizer (-7‰ to +7‰), animal and septic waste (+2‰ to +25‰), and atmospheric deposition (meteoric precipitation containing $NH_4^+$ and $NO_3^-$) (-7‰ to +8‰) (Kreitler 1979; Aravena and Robertson 1998; Kendall 1998; Einsiedl and Mayer 2006; Choi et al. 2007; Kendall et al. 2007). When one considers these ranges, in addition to the given amount of fractionation of the aquifer as unknown, it is often better to use an additional isotope to help refine the accuracy of the analysis. To this effect, the heavy isotope of oxygen ($^{18}O$) of nitrate is often used. The typical ranges for different $\delta^{18}O$-$NO_3$

sources in groundwater are as follows: natural and cultivated soil and animal/septic waste nitrates range from -15‰ to +15‰, while inorganic fertilizers range from -15‰ to +25‰, and atmospherically deposited nitrate ranges from +20‰ to +95‰ (Kendall 1998; Einsiedl and Mayer 2006; Kendall et al. 2007).

Typically, $\delta^{18}O$ and $\delta^{15}N$ values for nitrate are used when analyzing for contaminants and sourcing of nitrate pollution. This dual approach allows for more accuracy and helps mitigate the effects of the $\delta^{15}N$ value overlap caused by fractionation (Aravena and Robertson 1998; Fukada et al. 2004; Einsiedl et al. 2005; Dähnke et al. 2008; Pellerin et al. 2009; Wankel et al. 2009; Xue et al. 2009; Yuan et al. 2012). Natural fractionation occurs in karst aquifers, water, and soil when $N_2$ and $N_2O$ leave the system through the process of denitrification, which causes an $\delta^{15}N$ value similar to animal and septic waste values (Panno et al. 2001; Xue et al. 2009). When pairing the $\delta^{18}O$ values with the $\delta^{15}N$ values, however, one should be able to compensate for that discrepancy, because the $\delta^{18}O$ values should not be affected by denitrification (Böttcher et al. 1990; Kendall 1998). This allows for a more accurate tracing of the nitrate sources (see Figure 2.3).

The importance of nitrogen to algal growth is well established; however, there exists some debate on the importance of nitrogen in freshwater in relation to phosphorus (Elser et al.1990; 2007; Schindler et al. 2008; Sterner 2008). Nitrogen to phosphorus ratios have shown to be important in varying degrees in relation to the composition of the ecosystem in which they occur (Schindler 1977; Paerl et al. 1995; Albertin 2009). Laboratory experiments have even shown that genera of algae respond differently to various sources of nitrogen (Berman and Chava 1999). Nitrogen's importance in karst-fed freshwater systems is established, with most of the primary research focused on Florida's karst aquifers (Albertin 2009; Tucker et al. 2014). Few studies have examined the nitrogen loading in Kentucky lakes (Moses and Barker 2014) and no published studies have examined the sources of nitrogen in Kentucky's karst-fed waterways using isotopes.

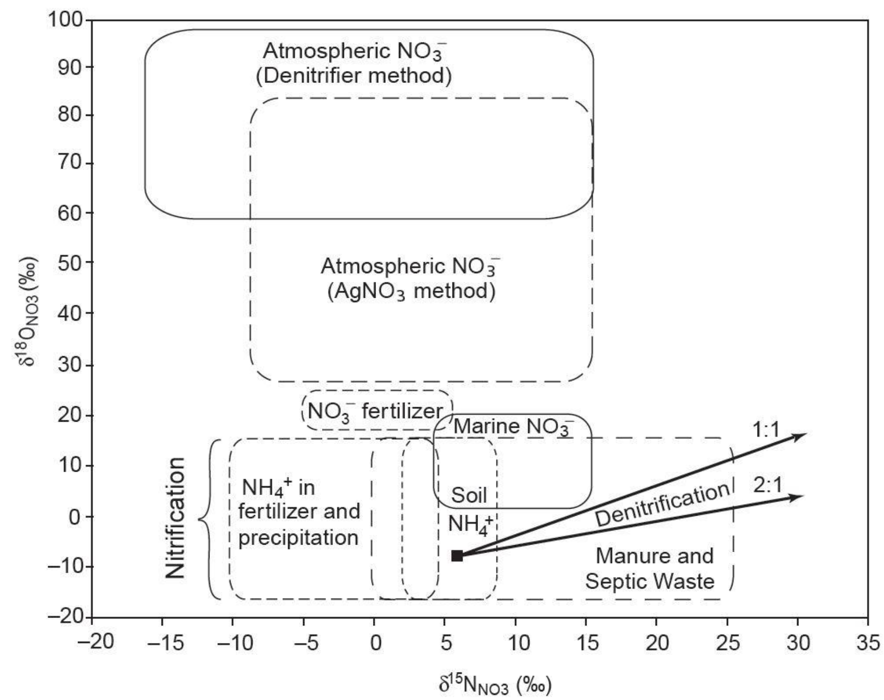



Figure 2.3. Interpreting $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ with a graphic mixing model. The graph shows where the normal distribution of $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values when they are plotted against each other and what the source $NO_3$ of those samples. Source: Kendall et al. (2007).

Phosphorous is the most widely studied element in water systems (Wetzel 2001). Ever since Schindler (1977) published his experiments on whole lake fertilization, phosphorus is widely held as the primary limiting nutrient source in freshwater systems. Phosphorus is important in the formation of DNA, RNA, ATP, and even the phospholipids that make up cell membranes. Phosphorus is so reactive that it does not occur naturally as a free element on Earth. In terms of the phosphorous cycle, this means that not much of it is precipitated into the environment. It is this aspect of the phosphorus cycle that humans have greatly impacted. In inhabited areas, larger amounts of phosphorous are found to precipitate from the atmosphere than in natural areas (Kortmann 1980; Gibson et al. 1995; Newman 1995; Tipping et al. 2014). Other sources of phosphorous enter freshwater systems via groundwater and runoff. Phosphorous readily bonds with bicarbonate and, as a result, mobile, reactive phosphorous is not found in high concentrations in groundwater or in hard-water lakes; however, more questions about the effects of subsurface waters and phosphorous contributions to eutrophication are starting to be investigated (Alloush et al. 2003; Holman et al. 2008). In some places, the saturation of phosphorous in the groundwater is high enough to be of concern; however, recent studies have attempted to link the motility of it in the groundwater of karst regions and address how it may play a role in the eutrophication of surface waters (Behrendt and Boekhold 1993; Sims et al. 1998; Holman et al. 2008; Assegid et al. 2014). In many freshwater systems, phosphorous plays a major role in algal bloom formation and growth (Jones and Bachmann 1976; Schindler 1977). Isotopic tracing of phosphorous has proven to be difficult and, as a result, oxygen isotopes are used to trace phosphate sources (Markel et al. 1994; Paytan and Mclaughlin 2012). Due to this difficulty, it is also beneficial to examine other contaminants, like fecal bacteria, when trying to identify phosphorous pollution sources. No studies exist that examine the levels and sources of phosphorous coming from karst outputs in central Kentucky lakes.

Micronutrients also play a role in eutrophication, especially in highly stratified bodies of water with many trophic states, such as reservoirs. Iron (Fe) is one of the more examined elements in this regard, as it is important to nearly all life. Iron is necessary for

$CO_2$, $O_2$, and $N_2$ fixation through the use of siderophores. Iron also plays a role in limiting production in freshwater systems in conditions where iron is in low abundance (Wurtsbaugh and Horne 1983; Wilhelm and Trick 1994). No studies have examined the effects or abundance of micronutrients in Kentucky's karst-fed waterways with respect to HABs. In other karst-fed systems, trace elements have been found to play a role in eutrophication (Sterner 1994), and some work has been done in Florida where nutritent loading of karst springs and surface waters is a rampant issue.

### 2.4 Groundwater Contaminants

A major source of nutrient contamination is the runoff from agriculture operations and wastewaters. These wastewaters are often caused by leaking septic tanks and wastewater effluent runoff and generally contain large amounts of fecal coliform and *E. coli* bacteria. Fecal coliform and *E. coli* bacteria pollution are a primary concern of water resource managers because the bacteria are dangerous to humans. Both bacteria can cause serious health issues if they are ingested or passed into the blood stream. As a result, total population counts of these bacteria in samples are used as a measure of water quality (de Brauwere et al. 2014).

Fecal coliform and *E. coli* bacteria can also be used to help determine the sources of nitrate pollution. An established connection exists between fecal bacteria population levels and the amounts of nitrate in groundwater, allowing for one to discern the effects local agriculture and urban sewage disposal practices have on the local groundwater (Kelly et al. 2009). The DNA of *E. coli* is used to trace the sources of nutrient pollution in groundwater systems (Katz and Griffin 2008). Fecal coliform and *E. coli* bacteria also move easily through karst systems, where the surface waters have direct access to the subsurface waters and septic lines can bleed into karst conduits for rapid transit into the groundwater system and exit at major springs, which often feed surface water bodies (Ryan and Meiman 1996; Ford and Williams 2007; Vanderhoff 2011).

In compliance with the Federal Water Pollution Control (or Clean Water) Act of 1972, the Kentucky Division of Water (KDOW 2013a) is required to make a biennial water quality report that summarizes the conditions and quality of Kentucky's waterways. The Division of Water's study found that fecal coliform and *E. coli* bacteria are the number one pollutant of Kentucky waterways (KDOW 2013b). When a waterway incurs too much of any one contaminant, like *E. coli*, nitrates, or phosphates, then the stream is placed under a Total Maximum Daily Load (TMDL) program by the Environmental Protection Agency (EPA) under section 303(d) of the Clean Water Act. This program works to institute guidelines for cleaning up the impaired waterway by setting the daily loadings of contaminants, establishing what a healthy stream's maximum limit would be, and helping set policies and regulating the contaminants for the waterway under the Clean Water Act (USEPA 2014). Nitrate isotopes alone cannot separate animal waste sources from human sources; however, when one tests for the presence of additional anthropogenic markers, such as caffeine and other bacterial markers, then some delineation can be achieved (Seiler et al. 1999; Katz and Griffin 2008; Zhang et al. 2014). Nitrate isotopes can delineate between mammal waste and commercial fertilizers; thus, it follows that these parameters and bacteria concentrations should be monitored. When bacterial monitoring is paired with nitrogen isotope tracing, this can be used as an effective detection and monitoring method for human septic and animal waste pollutant indicators and tracers when monitoring water quality.

### 2.5 Freshwater Harmful Algal Blooms

A HAB is a bloom of algae or blue-green algae (cyanobacteria) in response to a change in nutrient levels, temperature, solar radiation, zooplankton populations, or some other influence that gives the algae or cyanobacteria a competitive advantage (Wetzel 2001). HABs have been of concern for water resource managers and limnologist in North America since the mid-1800s, with increasing suspicion that humans play a major role in their formation and frequency of occurrence (Schindler and Vallentyne 2008; Taranu et al. 2015). Not all of the interactions between humans and HABs are fully understood, but the Centers for Disease Control and Prevention (CDC 2012) classifies them as a threat to human health and safety, due to the toxins they can produce at harmful levels that can affect humans and animals. HABs recently regained the public's attention with the large cyanobacteria bloom in Lake Erie (Panek 2012), and now are prevalent around the world, including in all 50 U.S. states, thus creating a global problem that may be linked to several overlying factors. The term "harmful algal bloom" is used as a blanket term to cover both nuisance levels of algae blooms and cyanobacteria blooms, which are not algae, nor plants, but rather prokaryote bacteria. Green algae can be broken down further into the phyla *Chlorophyta* (green algae), *Dinophyta* (dinoflagellates), *Cryptophyta*, and *Chrysophyta* (Reynolds 1984). All of the algae blooms are known to cause water quality problems such as taste, odor, aesthetics, as well as cause hypoxia of the water surrounding the bloom. It is also common for green algal blooms to be followed by cyanobacterial blooms (Paerl et al. 2001; Kennedy 2016).

Of all the types of algae, only the dinoflagellates (see Figure 2.4a) are known to produce toxins in freshwater systems, and blooms of this phylum are often referred to as "freshwater red tides" (Reynolds 1984). These algae bloom in conditions where the body of water is highly stratified, rich in nitrogen and phosphorous, and exposed to high amounts of solar radiation. As a result, reservoirs with anthropogenic sources of nutrient loading are at risk (Nakamoto 1975). Both green algae and cyanobacteria occur naturally across the globe and in all aquatic habitat types; however, they have recently begun to increase in frequency as the global climate warms and anthropogenic influence on fresh waterways increase (Paerl and Huisman 2008; Paerl and Paul 2011; O'Neil et al. 2012; Paerl 2014). Only in eutrophic ecosystems are blooms guaranteed to become a problem. When algae blooms in a eutrophic lake, it tends to create an environment of hypoxia, which kills off most of the grazers (zooplankton) that feed on the algae and cyanobacteria, thus allowing cyanobacteria to reproduce unchecked. Then, the cyanobacteria consume all the available phosphorus, starving out the algae and then feasting on their phosphorous rich remains, giving the cyanobacteria another source of phosphorous (Currie and Kalf 1984). When cyanobacteria rapidly reproduce in this manner, they form cyanobacterial HABs (CyanoHAB).

Cyanobacteria are some of the oldest known life forms on the planet. They have evolved to live in many conditions, environments, and ecosystems and naturally occur nearly everywhere. Cyanobacteria are so prevalent and diverse that researchers generally only identify them by their genera. These primitive life forms are of concern because they have the ability to produce toxins, commonly known as cyanotoxins. Little is known or understood about what conditions are needed for a bloom to produce toxins, although understanding is rapidly growing. Within the several taxa of naturally occurring cyanobacteria, toxin producing and non-toxin producing species exist and are relatively undistinguishable visually; however, it stands to reason that with the increase of a bloom's size, the probability of toxin production would also increase. These toxins can be released in a high-enough concentration to be harmful to wildlife and humans. Of the many Lake Erie *Lyngbya wollei* based HABs (see Figure 2.4b) that have occurred repeatedly since 1970, most have exhibited these problems (Panek 2012).

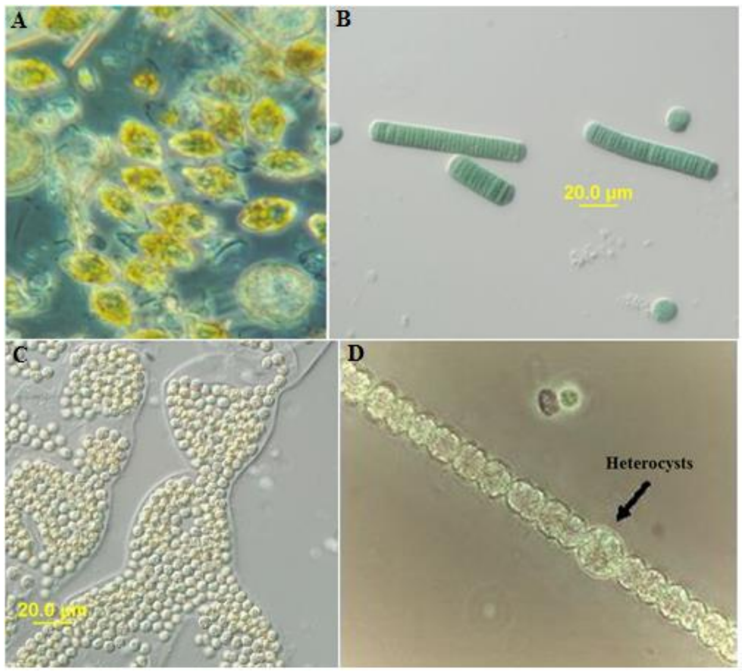



Figure 2.4. Electron microscope pictures of potentially harmful algae. A) Dinoflagellates B) *Lyngbya ssp.* C) *Microcystis ssp.* D) *Anabaena ssp.* Source: Paerl et al. (2001); USGS (2013a).

Cyanobacteria produce three types of toxins: hepatotoxin, dermatotoxins, and neurotoxins. These toxins all target different systems and there are a few set standards and several methods to measure for them in freshwater systems (USACE 2013; KDOW 2013c; 2014; Farrer et al. 2015). In freshwater systems, hepatotoxins and dermatotoxins are more commonly produced. In 1931, HABs of *Microcystis spp.* (see Figure 2.4c) on the Ohio and Potomac rivers caused between 5,000 and 8,000 reported cases of illness and the toxins remained in the water even after being subjected to precipitation, filtration, and chlorination (Tisdale 1931).

Recently, a few reported cases of human illness and dog deaths were attributed to cyanotoxins from *Microcystis spp.* toxins in Kansas (Trevino-Garrison et al. 2015). The cyanobacteria that produce hepatotoxins are rarer in occurrence; the dermatotoxin producing microcystins are common and occur worldwide (Sivonen 1996). In some rare cases, freshwater cyanotoxins have been known to make mussels toxic for human consumption through the process of bioaccumulation (Eriksson et al. 1989). Recently, bioaccumulation was documented in fish muscle tissue (Drobac et al. 2016). *Anabaena ssp.* (see Figure 2.4d) is a naturally occurring cyanobacteria in Kentucky lakes and has been known to produce anatoxin-a, which is known to cause fast deaths and is found to be fatal even at low concentrations (Negri and Jones 1995; FTN Associates 2002). One of the most difficult CyanoHABs to see occurring with the naked eye are those dominated by *Cylindrospermopsis*, which evenly distribute throughout a water column and only make the water appear cloudy. This type of cyanobacteria has been known to produce the cyanotoxin cylindrospermsin, which is a toxin that is typically released into the water by live bacteria, as opposed to the other cyanotoxins, which are typically released only upon cell death (USEPA 2015). With the way these toxins are released and transported in freshwater systems, animals and people downstream of these CyanoHABs are also at risk of injury (USGS 2012). These toxins pose an inherent dangers to humans, so the WHO created safety standards and guidelines addressing cyanobacteria in water resources (Chorus and Bartram 1999, WHO 2003) and the USEPA has released recommendations for drinking water standards (USEPA 2012)

In 2013, the USACE partnered with the Kentucky Division of Water (KDOW) after the USACE detected cell count levels of cyanobacteria considered dangerous by WHO standards (KDEP 2013; KEEC 2013). The WHO defines the danger levels based on cells per milliliter of water. Waters that contain 20,000 cyanobacterial cells per milliliter are defined to be low risk from which short-term skin irritation and gastrointestinal illness may develop. Waters containing 100,000 or more cyanobacterial cells per milliliter are of moderate risk, where risks of long-term illnesses are present (Chorus and Bartram 1999; WHO 2003). In addition to the cell counts, the WHO also recommends that the waters be monitored for cyanotoxins as well, particularly microcystins, and that advisories should be issued when concentrations are above 10 μg/L, or 10 ppb (Health Canada 2002, WHO 2011). As of November 29, 2014, all five of the USACE's reservoirs in Kentucky were found to have moderate levels of cyanobacteria, warranting them to issue water safety warnings (USACE 2014a). The KDOW issued additional water safety warnings for nine additional lakes and reservoirs across the state (KDEP 2014). Recently, a shift in focus has occurred in monitoring of cyanobacteria cell counts to the presences and concentrations specific cyanotoxins (Farrer et al. 2015). Many states have adopted this method of monitoring but, as of June 2015, the KDOW switched to monitoring exclusively for the presence/absence of cyanotoxins. (KDOW 2014). This is a departure from every other method for HAB monitoring in the U.S. and across the globe (Deutschland Umweltbundesamt 2012).

CyanoHABs pose a threat to human safety and water quality. Many studies have shown that cyanobacteria, on the whole, tend to bloom in the presence of excessive nitrogen and phosphorous levels when the water system has high residence times and stratification (Stevenson et al. 2007); however, mixing dynamics and sediment loadings in freshwater reservoirs can cause light to be the limiting factor, which is a strong consideration given the climate and seasonally heavy precipitation that can increase sediment loading and turbidity. Studies indicate the occurrence of CyanoHABs is increasing in frequency and magnitude in response to climate change and anthropogenic nutrient loading (Paerl and Huisman 2008; O'Neil et al. 2012; Paerl and Paul 2011; USEPA 2013; Taranu et al. 2015; Mowe et al. 2016). Given the danger these blooms pose to water quality in Kentucky lakes, more research needs to be performed in regards to the causes and formation of CyanoHABs in karst-influenced surface water systems.

### 2.6 HABs in Karst Areas

Few studies exist that examine the role that karst environments play in relation to HABs. A case study was performed on three tributaries of Lake Marion, South Carolina (Williams et al. 2014). The tributary that had the highest flow was a cave spring. The focus of the study was to evaluate the discharge of the tributaries and the effect of nitrogen and phosphorous loading on a nearby reservoir-like embayment. The study found a majority of the embayment's nitrogen loading came from the cave spring and determined the nitrogen to be primarily from surface sources. The researchers were not able to decipher those sources with qualitative accuracy (Williams et al. 2014).

Several studies exist that examine the impacts of nutrient loading on HABs located in and around spring outputs in Florida (Katz 2004; Stevenson et al. 2007; Albertin et al. 2011). The nitrate concentrations at the Rainbow Springs complex in Marion County, Florida, have been monitored since 1965 (Jones et al. 1996). Most of these studies have found inorganic chemical fertilizers to be one of the main culprits of nitrogen loading in the karst-fed waterways; however, no one has attempted to study spring-fed lakes in Kentucky in this manner. Kentucky's karst, climate, soil, terrain, and agricultural practices differ from Florida's as well, so the Floridian studies are only useful as references to Kentucky resource managers. There is a lack of literature in regards to monitoring karst inputs of reservoirs and their effects on HAB formation in Kentucky, which makes it difficult for the local water resource managers to address them.

Identifying the sources of the nutrient loading and establishing the role karst inputs play in HAB formation are essential for water resource managers to determine if there are flow management options or land-use BMPs that can alleviate HAB development and assist the partner regulatory agencies in identifying problematic areas in the watersheds that need to be addressed. A combination of isotopic and bacterial analyses would be the most effective way to source the anthropogenic nutrient and contaminant pollution in a karst system, which has not been done in Kentucky's karst-fed reservoirs. This pioneering study could help set the stage for more research in other karst areas across the globe, as well as help establish best management practices that have the potential to save lives, mitigate economic damage, and improve the water quality of people affected by HABs in those karst areas.

## Chapter 3: Sourcing and Dynamics of Karst Hydrologic Inputs on Harmful Algal Bloom Occurrences in Kentucky Lakes

### 3.1 Introduction

Almost half of Kentucky is underlain by a well-developed karst aquifer/landscape system in which water resource access and protection challenges are common. Surface water supplies are often limited, groundwater is typically vulnerable to contamination, and even basic delineation of aquifer recharge areas requires specialized methods (Ford and Williams 2007). Karst landscapes are formed through a process of dissolution, through which carbonate rocks are typically dissolved by a slightly acidic solution of carbonic acid. The origin of this acid is mostly from soil $CO_2$ derived from plant and microbial respiration, which is dissolved into percolating groundwater (Palmer 2007). The resulting landscape is limited in surface water, due to the complex, internal drainage systems that develop over time.

Figure 3.1 illustrates a hydrological map that includes karst features and indicates that few surface water features are found within a sinkhole plain. Karst landscapes facilitate the rapid transport of water and contaminants from the surface to the subsurface through karst features, such as sinkholes, aquifer, conduits, and caves (Hess and White 1988; 1993). The surface water that does exist is often in the form of rivers. Many of these rivers are fed by springs and serve as base level for their drainage areas. Much of the rainfall in Kentucky is drained internally through its karst system. Due to this internal drainage, Kentucky has few major, naturally occurring lakes. Most importantly, the lakes that do exist also provide a drinking water source for several communities. In particular, Nolin River Lake and Rough River Lake, located in Kentucky and created under the Flood Control Act of 1938, are important and heavily used recreational lakes that also

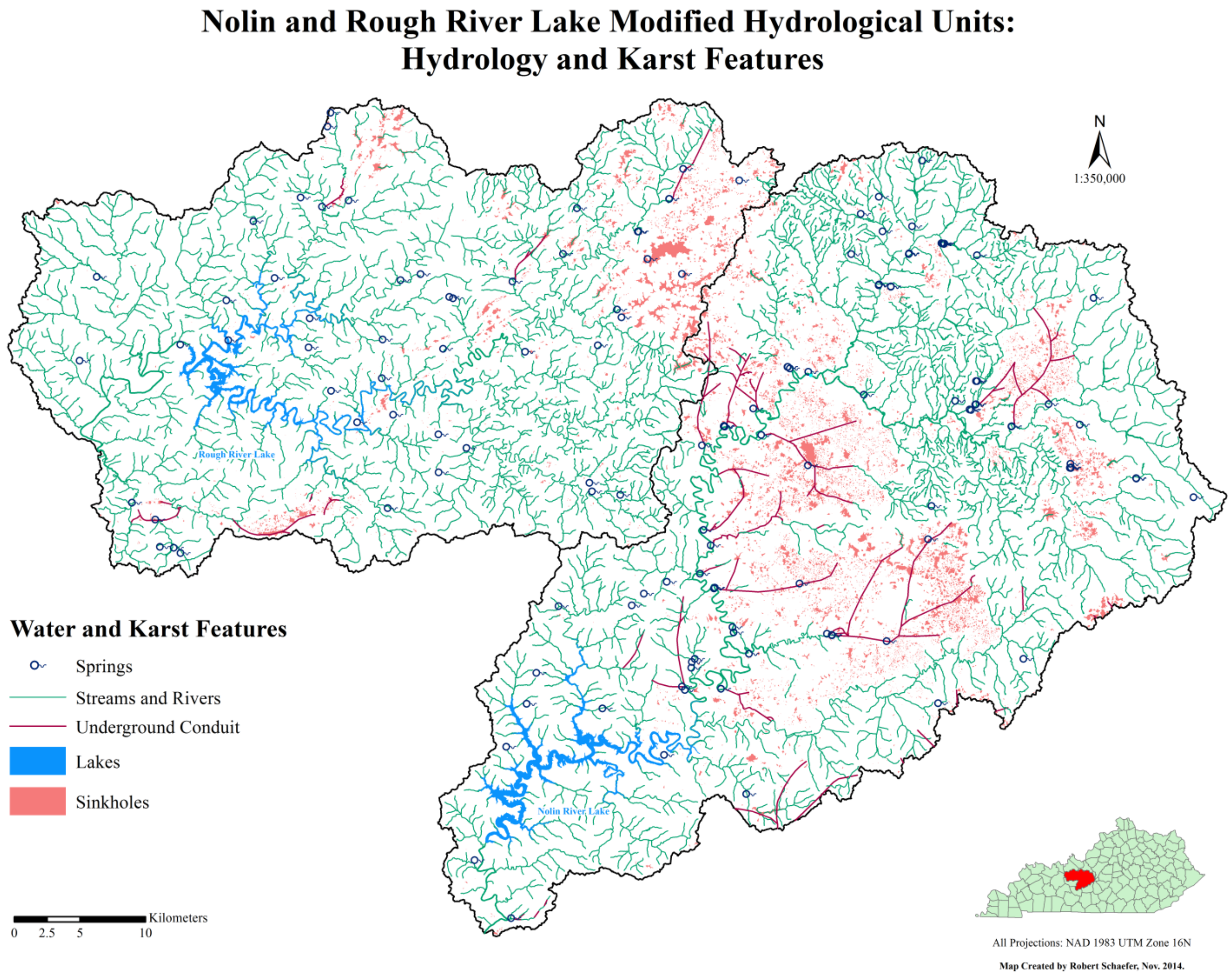



Figure 3.1. Hydrology and karst features of Rough River and Nolin River Lakes' modified hydrological units. Note that the surface water features in the sinkhole plains are very limited. The underground conduit paths are generalized and not representative of the true paths; they were found with dye tracing. The mappedarea (in red) is found in the reference map of Kentucky. Source: Created by the author from KGS (2003; 2004); Tiger/Line (2010); USGS (2013b).

serve several of the aforementioned functions, particularly flood mitigation (Grubbs and Taylor 2004).

Recently, freshwater harmful algal blooms (HABs), predominantly consisting of cyanobacteria, have been occurring in the Rough River Lake and Nolin River Lake reservoirs and may be caused by nutrient loading introduced through karst hydrologic inputs (Figure 3.2). Most notably, these blooms have occurred during winter at both lakes. These blooms present an issue for the local stakeholders as there exist few data regarding the cause of cyanobacterial HABs (CyanoHABs) within the context of karst groundwater-fed riverine systems. It should also be noted that a large sinkhole plain exists within the watersheds of both Nolin River Lake and Rough River Lake, although it is a more dominant land feature within the Nolin River Lake watershed (Figure 3.1), and contributes most of the recharge to the regional karst aquifer.

Further complicating the issue is the nature of karst landscapes; they are a complex system in which tracing hydrogeologic pathways and watershed delineation is difficult (Palmer 2007). Without extensive and costly dye tracing, the only way to trace sources of the nutrient pollution is to use alternative methods. In the case of karst groundwater, the heavy isotopes $^{18}O\text{-}NO_3$ and $^{15}N\text{-}NO_3$ are proven to be effective in identifying the origins of nitrate contamination (Kambesis 2007; Kendall et al. 2007; Albertin et al. 2011; Williams et al. 2014).

Figure 3.2. Cyanobacterial HABs that formed in Nolin River Lake on 9/17/15. Source: USACE (2015).

Nitrate isotopic tracing is done through measuring the ratio of the less abundant, nitrogen-heavy isotope ($^{15}N$) to that of the more abundant, lighter one ($^{14}N$). This ratio is expressed as a per mil (‰) value of $\delta^{15}N$. The typical range of values for $\delta^{15}N$ in groundwater for different sources are soil nitrate (-3‰ to +14‰), inorganic fertilizer (-7‰ to +7‰), animal (including liquid and solid manure) and septic waste (+2‰ to +25‰), and atmospheric deposition (meteoric precipitation containing $NH_4^+$ and $NO_3^-$) (-7‰ to +8‰) (Kreitler 1979; Aravena and Robertson 1998; Kendall 1998; Einsiedl and Mayer 2006; Choi et al. 2007; Kendall et al. 2007). When one considers these ranges, in addition to the given amount of fractionation of the aquifer being unknown, it is often better to use an additional isotope to help refine the accuracy of the analysis. In the case of nitrates, the heavy isotope of the oxygen in nitrate ($^{18}O$-$NO_3$) is often used to this effect (Aravena and Robertson 1998; Fukada et al. 2004; Einsiedl et al. 2005; Dähnke et al. 2008; Pellerin et al. 2009; Wankel et al. 2009; Xue et al. 2009; Yuan et al. 2012). The typical ranges for different $\delta^{18}O\text{-}NO_3$ sources in groundwater are as follows: natural and cultivated soil and animal/septic waste nitrates range from -15‰ to +15‰, while inorganic fertilizers range from -15‰ to +25‰, and atmospherically deposited nitrate ranges from +20‰ to +95‰ (Kendall 1998; Einsiedl and Mayer 2006; Kendall et al. 2007).

Identifying the sources of nitrate contamination can serve as a proxy for finding the sources of the other major nutrient polluters. Most of the sources of nitrate are also sources of the main limiting nutrient in freshwater systems, which is often phosphorus (Schindler 1977). Phosphorus typically exists as phosphate ($PO_4$) and is often the main limiting nutrient in freshwater systems; however, nitrate is thought to also play a role in freshwater systems (Elser et al.1990; 2007; Schindler et al. 2008; Sterner 2008); thus, nitrate sourcing can be used as a proxy for phosphate sourcing. Other nutrient proxies that can be used are indicator fecal bacteria, such as *Escherichia coli* (*E. coli*). These bacteria can be used to supplement the nutrient concentration data by shedding some light on the bioactivity of the water, as well as showing where some of the septic/animal waste constituents of the nutrient contamination may have originated.

CyanoHABs are not only threatening the local ecosystems of Rough River Lake and Nolin River Lake, but also threating the livelihoods and viability of the local communities. Currently, a method does not exist for monitoring or prevention of CyanoHABs within the context of a karst landscape and freshwater system. It is the goal of this study to be one of the first to establish monitoring parameters and data collection methods to determine the level of influence karst hydrological inputs have in CyanoHAB formation. In addition, this research focuses on measuring the concentrations of anthropogenic contamination and nutrient pollution entering the lakes through the karst tributaries. The tracing of these nutrients through heavy $NO_3$ isotopes was employed in an attempt to delineate the sources of the nutrient pollution, along with multiple geochemical and climatic parameters. The final goal of this study is to help develop a method for monitoring CyanoHAB formation as to assist in the establishment of a set of best management practices (BMPs) for CyanoHAB prevention, allowing for directed plans of action to be developed.

### 3.2 Study Area

Central and south-central Kentucky are home to one of the most biologically diverse river systems in North America, the Green River, as well as to several listed, endangered, and threatened species (USACE 2011). Both Nolin and Rough River lakes drain into the already eutrophic Green River, which flows through a large, well developed karst landscape (Penick et al. 2012). Kentucky waterways are notably difficult to manage due to the karst nature of the region (Grubbs and Taylor 2004). Most of south-central Kentucky is part of a large formation of limestone known as the Pennyroyal Plateau, which is mostly made of heavily karstified limestones (Palmer 2007). The drainage basins for both Nolin River Lake and Rough River Lakes are part of this plateau (Figure 3.3). Both of the upper areas of the drainage basin stratigraphy for each site are composed mostly of St. Louis and St. Genevieve Limestone, while the lower drainage basin stratigraphy for both sites is mostly made of younger Chesterian age rocks (KGS 2002; USGS 2005) (Figure 3.3). Although Rough River Lake and Nolin River Lake are in heavily karstified central Kentucky, both have differing qualities, surrounding landuses, and drainage basins.

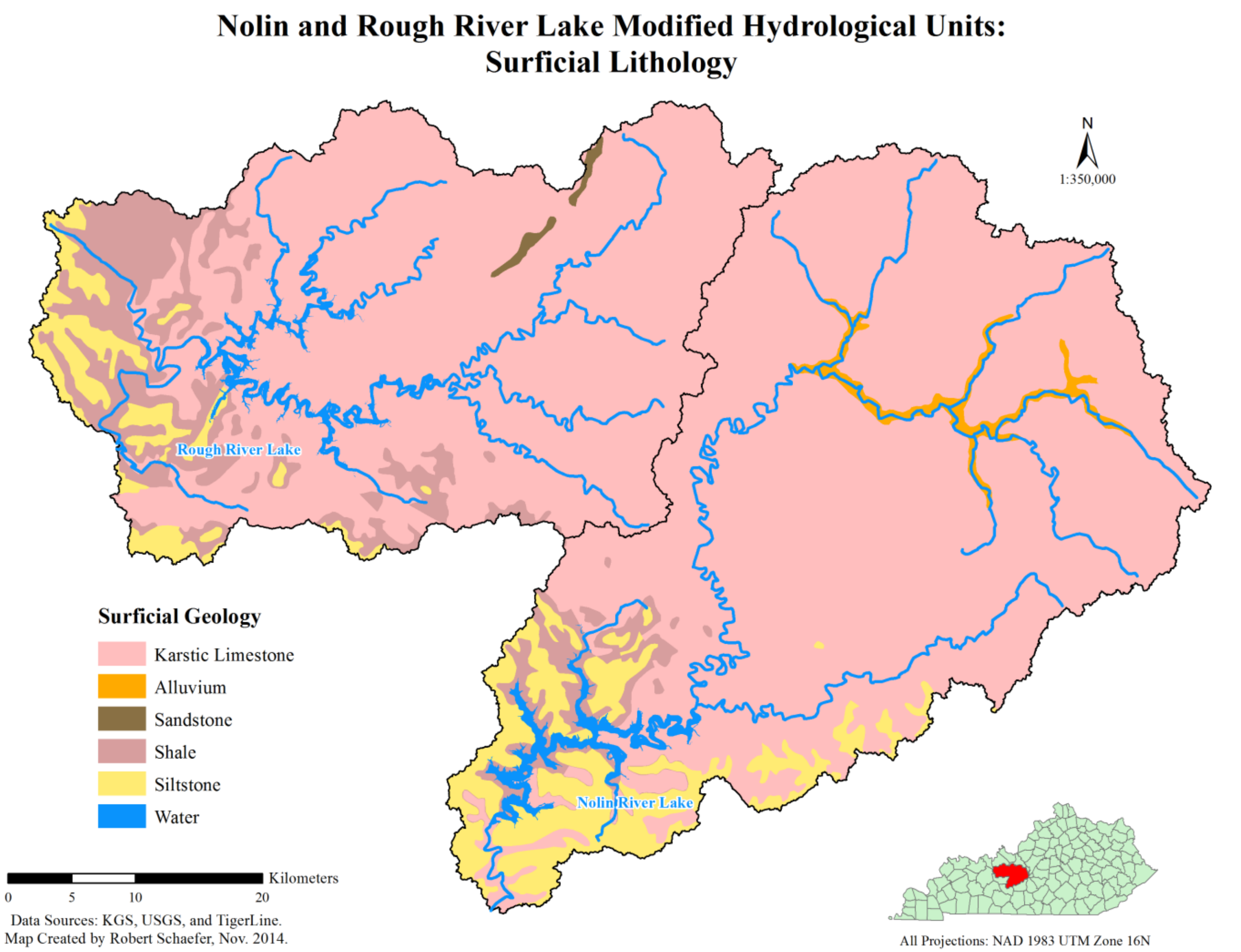



Figure 3.3. Surficial lithology of the Rough River Lake and Nolin River Lakes' modified hydrological units. The units are dominated by karstified limestone and although the lakes' local lithology is alluvial, karstic limestone is underneath. As such, karst hydrological features are still found in and around the lakes, as seen in Figure 3.1. Source: Created by the author from KGS (2002); Tiger/Line (2010); USGS (2013b).

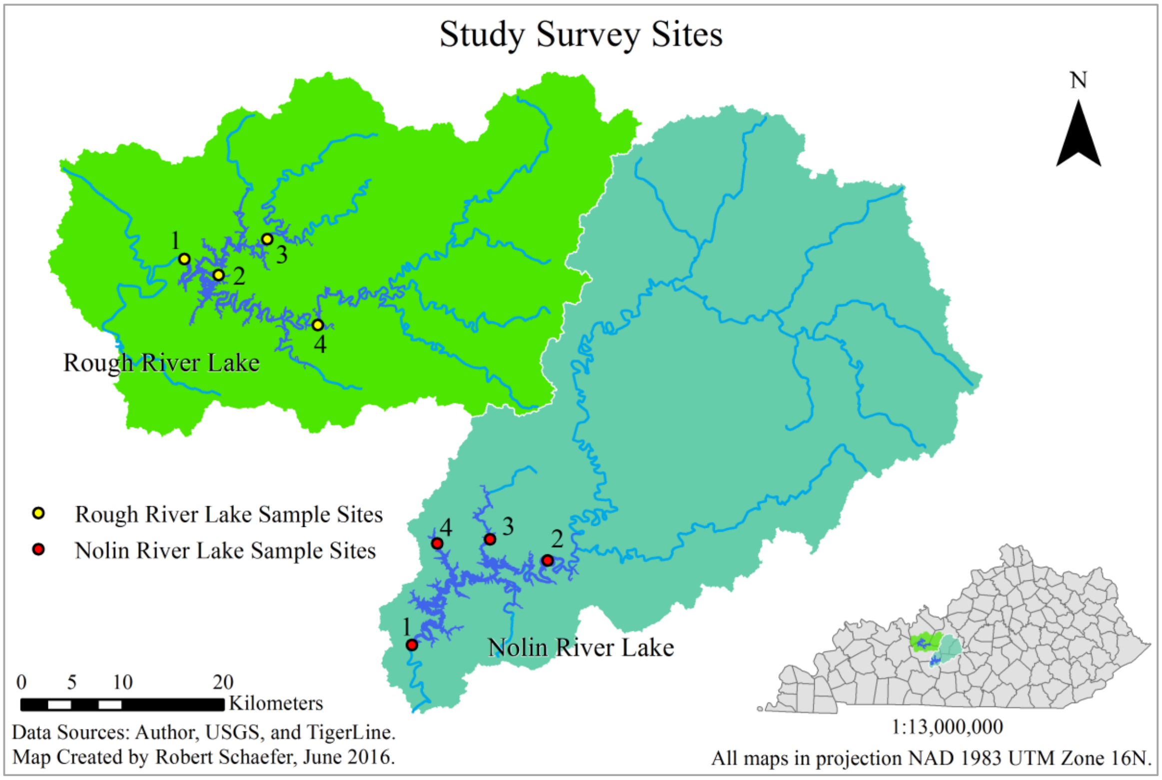



Figure 3.4. Study Survey Sites, with four sample sites per lake. Three sites within the lakes, representing the karst tributaries (Sites 2-4 at each lake) and one site in the tailwaters of each dam (Site 1 for both lakes). Rough River Lake's hydrological unit appears in yellow-green and Nolin River Lake's hydrological unit appears in blue-green. Both units are noted in their respective colors on the inset map.
Source: Created by the Author from TigerLine (2010); USGS (2013b).

Nolin River Lake was created by the U.S. Army Corps of Engineers (USACE) in 1963 by the building of an earthen dam as part of the Flood Control Act of 1938 (Grubbs and Taylor 2004). While the lake still performs this function, it now also serves as a water source for the local communities and a major source of recreation and income for the area. The lake varies in area from 1,169.5 hectares (11.7 km²) in the winter to 2,345 hectares (23.5 km²) in the summer (USACE 2014b).

The drainage basin of Nolin River Lake is mostly karstified limestone; however, the local lithology of the adjacent area is dominated by shale and siltstone (Figure 3.3). The siliciclastic rocks around the lake cause the runoff in the immediate area to go directly into the lake, although several karst hydrological outputs exist in the immediate area. These sources of groundwater largely exist in the form of several springs, and also include other karst formations within the lake, such as the blue hole in one of the northern tributaries near Iberia (see Figure 3.1 and Nolin Site 3 in Figure 3.4). A blue hole is a karst window that provides a direct exchange of groundwater and its dissolved constituents between the karst aquifer and a surface water body; in most cases, a blue hole is a submerged sinkhole. It should also be noted that, in reference to Figure 3.1, not all of the springs, blue holes, or sinkhole are represented on the map and that only the currently known features appear on the map. It is likely that many more karst surface features actually exist; in fact, several springs were observed during the course of the study in and around the lake that are not represented on the map due to their ephemeral visibility from changing lake levels.

The land coverage of the Nolin Lake hydrological unit is mostly agriculture at 48% coverage, and 44% of the land is covered by forest. Urban coverage makes up 6% of the hydrological unit's land surface and 2% is made up of water and wetlands (see Figure 3.5). The local areas around the lake are sparsely settled and the primary land-use in the immediate vicinity of the lake is agricultural. These agricultural practices are primarily focused on hay/pasture lands and large monoculture, row crop fields, with corn and soybean as the most prevalent (USGS 2011). These numbers were derived from USGS 2011 National Land Cover Database (NLCD) in combination with the modified hydrological units, because the exact drainage basins of any surface water in a karst landscape are often difficult to delineate.

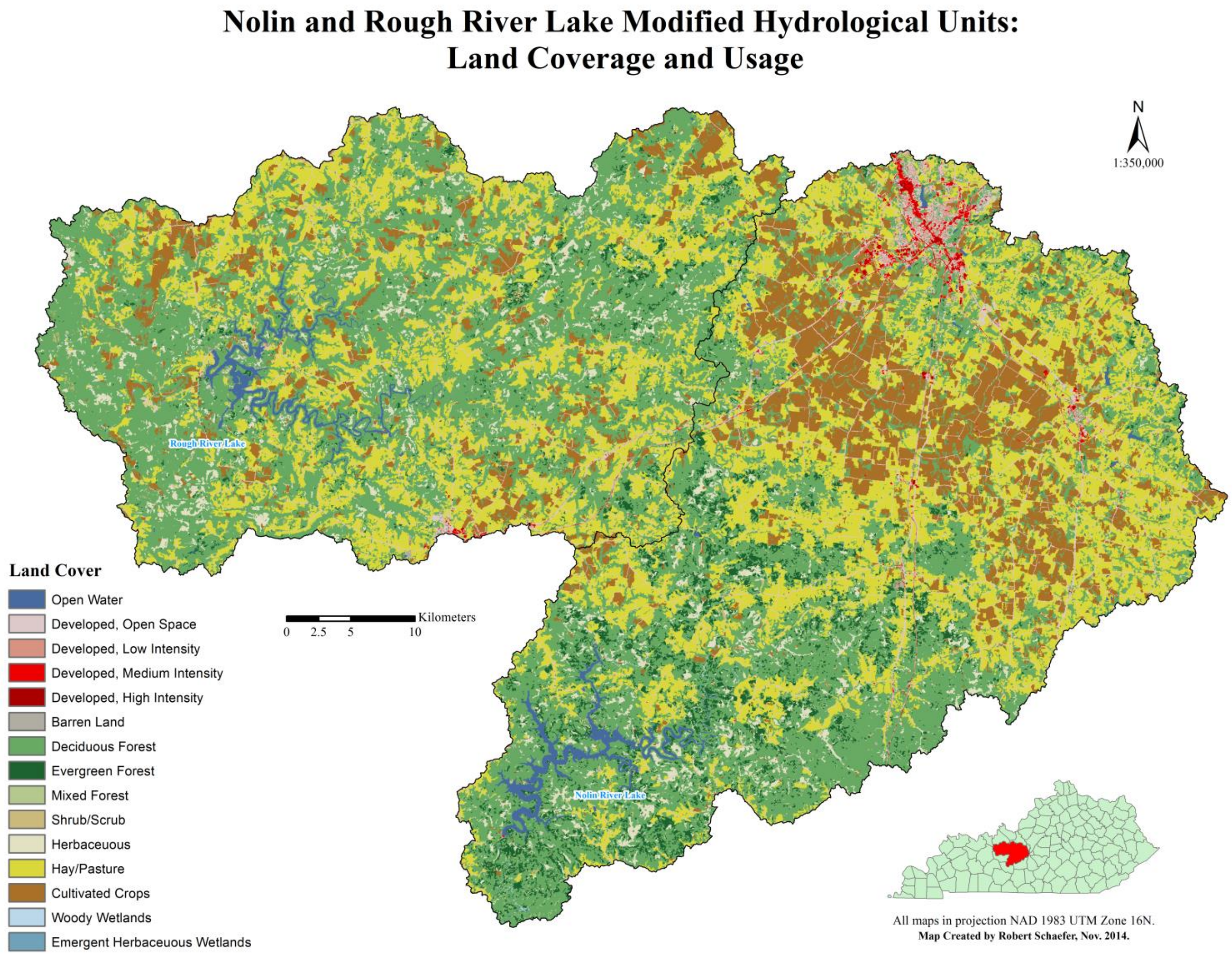



Figure 3.5. Land coverage of the Rough River Lake and Nolin River Lakes' modified hydrological units. The units have similar land coverage percentages; however, Nolin River Lake's unit has denser clusters of urban development, as well as 21% more area than the Rough River Lake hydrological unit. Source: Created by the author from Tiger/Line (2010); USGS (2011; 2013b).

Nolin Lake has several tributaries, but the two largest are Bacon Creek and Nolin River. Bacon Creek currently has a Total Maximum Daily Load (TMDL) for high concentrations of *E. coli* (KDOW 2013b). Nolin River flows through Hardin County and drains the Elizabethtown, Kentucky area. Billy Creek and Valley Creek, two of the tributaries that flow into Nolin River and drain Elizabethtown's city reservoir have TMDLs for eutrophication on record. With at-risk tributaries and the concentration of urban populace and agricultural practices in a sinkhole plain, Nolin River Lake is under continual risk for anthropogenic eutrophication and HAB formation. In fact, Nolin River Lake was placed under HAB advisories in both 2013 and 2014 (KDEP 2013; 2014).

Rough River Lake is located to the northeast of Nolin River Lake and is the other lake of interest for this study; it was also created by the USACE as part of the Flood Control Act in 1959. Rough River Dam is also earthen and is currently undergoing remediation for structural undermining occurring from the development of sinkholes and leakage, due to its construction on karst bedrock. The reservoir still serves as a water control structure, but also provides the drinking water for the local community and is a major source of income in the area through recreational use. Rough River Lake is 2,064 hectares (20.6 $km^2$) in size (USACE 2014c). Rough River Lake's size does not change drastically by season because its banks are much steeper than Nolin River Lake's banks, meaning that Rough River Lake has more of a seasonal depth change.

The local lithology of Rough River Lake is dominated almost solely by upper Mississippian-aged limestone overlain by a sandstone cap (Figure 3.3) (KGS 2002; USGS 2005). While the limestone in the area is prone to karstification, it is confined by sandstone, thereby causing the karst features to occur at lower elevations, where the sandstone has eroded away. The Kentucky Geological Survey (KGS) identifies a few blue holes that occur within the reservoir itself. Like the blue hole at Nolin River Lake, these provide a direct interface between the groundwater and the reservoir. The KGS and the Kentucky Speleological Survey also identify a few other karst inputs in the reservoir in the form of springs (KGS 2004) (Figure 3.1).

The USGS 2011 NLCD imagery indicates that the dominant type of land coverage of Rough River Lake’s hydrological unit is agriculture at 48% (see Figure 3.5). Forest covers 43% of the hydrological unit and urban development covers 7% of the land. Wetlands and water features occupy the remaining 2% (USGS 2011). These numbers seem similar to those of Nolin River Lake, but the total area for Rough River Lake’s hydrological unit is 21% smaller than Nolin River Lake’s hydrological unit. After a field visit, it was also observed that the immediate area surrounding the lake is under heavy development for subdivisions. These subdivisions are primarily using septic systems as their main means of waste disposal. Intensive and widespread agricultural production surrounds the lake just outside of its main floodplain, where the ground is more suitable for corn production than urban development.

Rough River Lake has five tributaries, the main ones being the Rough River main branch and Rough River North Fork. All of these tributaries drain karst areas dominated by limestone geology (Figure 3.3). None of the tributaries of Rough River Lake have a TMDL established. Rough River Lake’s blue holes provide direct recharge from groundwater and put it at risk for contamination from leaking sewage and agricultural processes occurring in the nearby rural communities and entering the groundwater.

Both Nolin River and Rough River Lakes share south-central Kentucky's temperate climate and fall under the Cfb designation of the Köppen climate model (Peel et al. 2007). The region is temperate with mild winters, warm summers, and no defined dry season. Most of the annual rainfall occurs in the spring with an annual average temperature of 11.6 °C to 15 °C (KCC 2014). These climatic conditions are ideal for HAB formation from the spring through to fall; however, Kentucky's winters are typically colder than most cyanobacteria can tolerate, which drives the need for a better understanding of from where the temperature influence may derive that causes warmer conditions during the winter.

### 3.3 Materials and Methods

HABs form under specific conditions, which can be monitored for and used as indicators for the potential threat they may pose to humans and wildlife. The eutrophic level of a lake is a good indicator of the threat of bloom formation and most measures of water quality are also indicators of HAB formation potential. These water quality parameters include dissolved oxygen (DO), turbidity (TU), temperature (Temp), specific conductivity (SpC), pH, and the concentrations of nutrients, such as nitrogen ions ($NO_3$, $N_2O$, $NH_3$), phosphate ($PO_4$), and other trace metals, all of which can also be used to assess the trophic level of the lake (Wetzel and Likens 1991; Yamamoto and Nakahara 2005; Paerl and Paul 2011; Paerl and Otten 2013). Tests for *E. coli* bacteria and the stable isotopes of nitrogen ($^{15/14}N$-$NO_3$) and oxygen ($^{18/16}O$-$NO_3$) in nitrate can be used to source and delineate nitrate contaminants contributing to HAB formation (Seiler et al. 1999; Kendall et al. 2007).

Before site selection commenced, historic HAB data, provided by the USACE, were first analyzed with use of ArcGIS for Desktop 10.2. These data were comprised of cyanobacterial cell counts collected during the latter half of 2013. Due to the lack of sampling guidelines in Kentucky at the time of the surveys, the duration of the study for each lake varied in length and frequency. The survey at Nolin River Lake was conducted on a monthly basis from July until December, 2013 (Figure 3.6). At Rough River Lake, the sampling was conducted weekly from July until September, 2013 (Figure 3.7). Both surveys highlighted the importance of monitoring CyanoHABs as the cyanobacterial cell counts were consistently higher than values deemed safe (200,000 cells/mL) by the World Health Organization (WHO). For Nolin River Lake, the consistently high counts even ranged into winter. The results of each survey were plotted on maps of each lake using the established USACE HAB sampling locations and then analyzed using kernel density estimation (KDE) to create "heat" maps (Figure 3.8a-b), where the darker areas of the map represent the areas of highest cell counts. These maps created a way to visualize the spatial and temporal aspects of the 2013 cyanobacterial blooming season, allowing for patterns to be seen and areas of interest to be selected for potential sample sites and future field visits. In addition to aiding site selection, the historic cell-count data also helped provide a list of cyanobacteria that were repeatedly blooming in large numbers, providing a list of target species/genera, with *Cylindrospermopsis raciborskii* dominating the counts throughout the 2013 sample periods for both lakes.

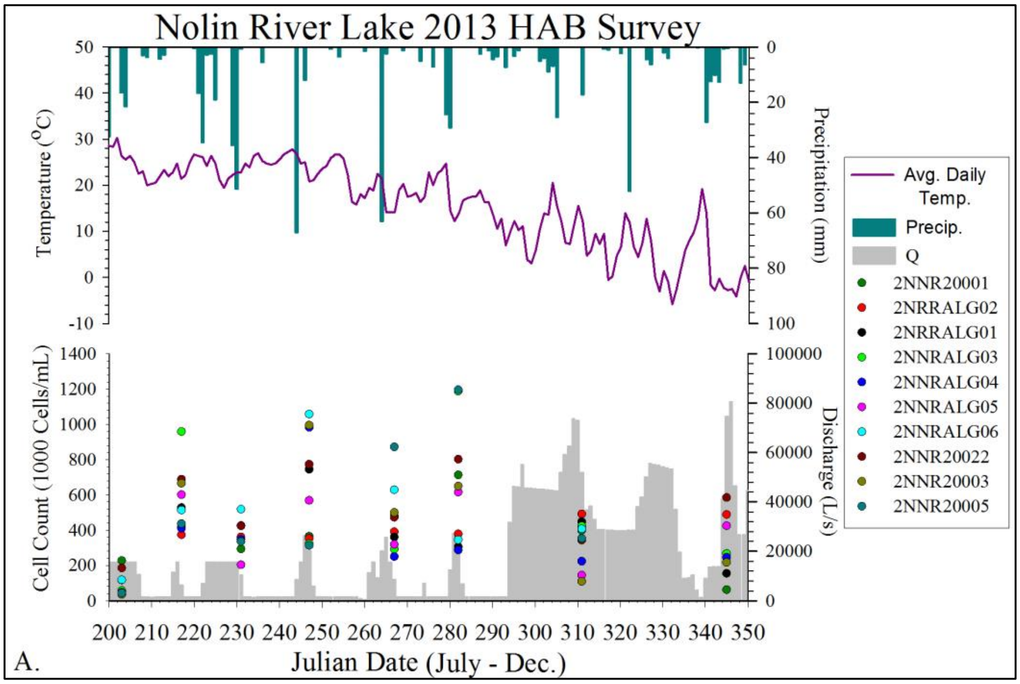



Figure 3.6. HAB survey and weather data for Nolin River Lake from July-Dec. 2013. Note, Nolin River Lake's cell counts in December are well above the 200,000 cells/mL that the WHO has deemed dangerous. Source: Created by the author from USACE, NWS/CoCoRaHS weather station data.

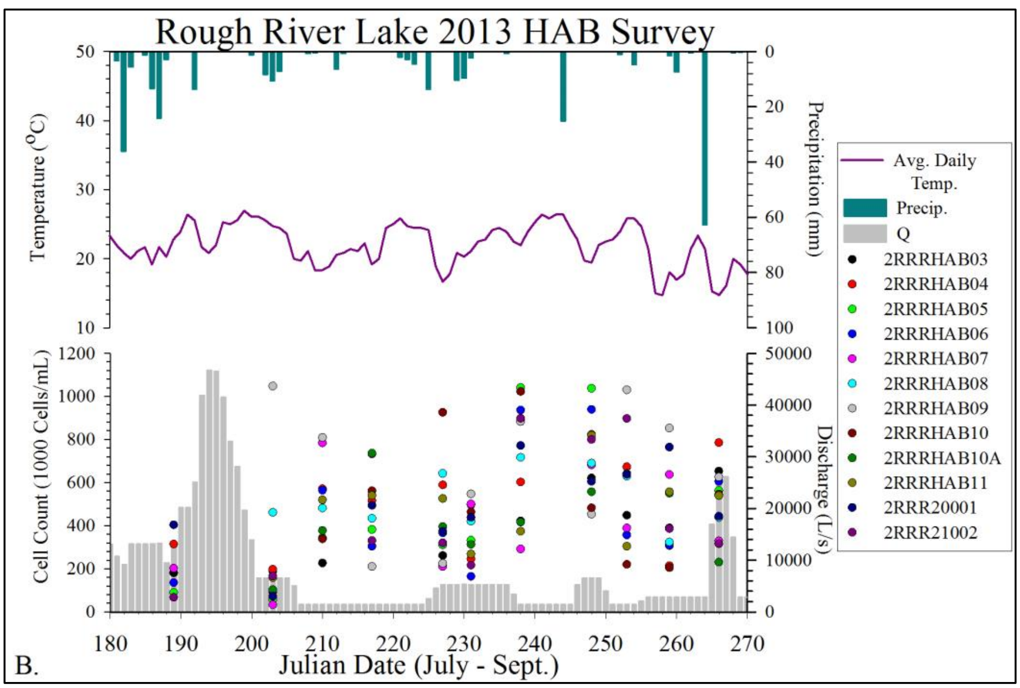



Figure 3.7. 2013 Cell count survey data from Rough River Lake. These are paired with the weather data from July-Sept. For Rough River Lake the data show some serious cell counts throughout the typical blooming season, with several blooms breaching 1,000,000 cells/mL.
Source: Created by the author from USACE, NWS/CoCoRaHS weather station data.

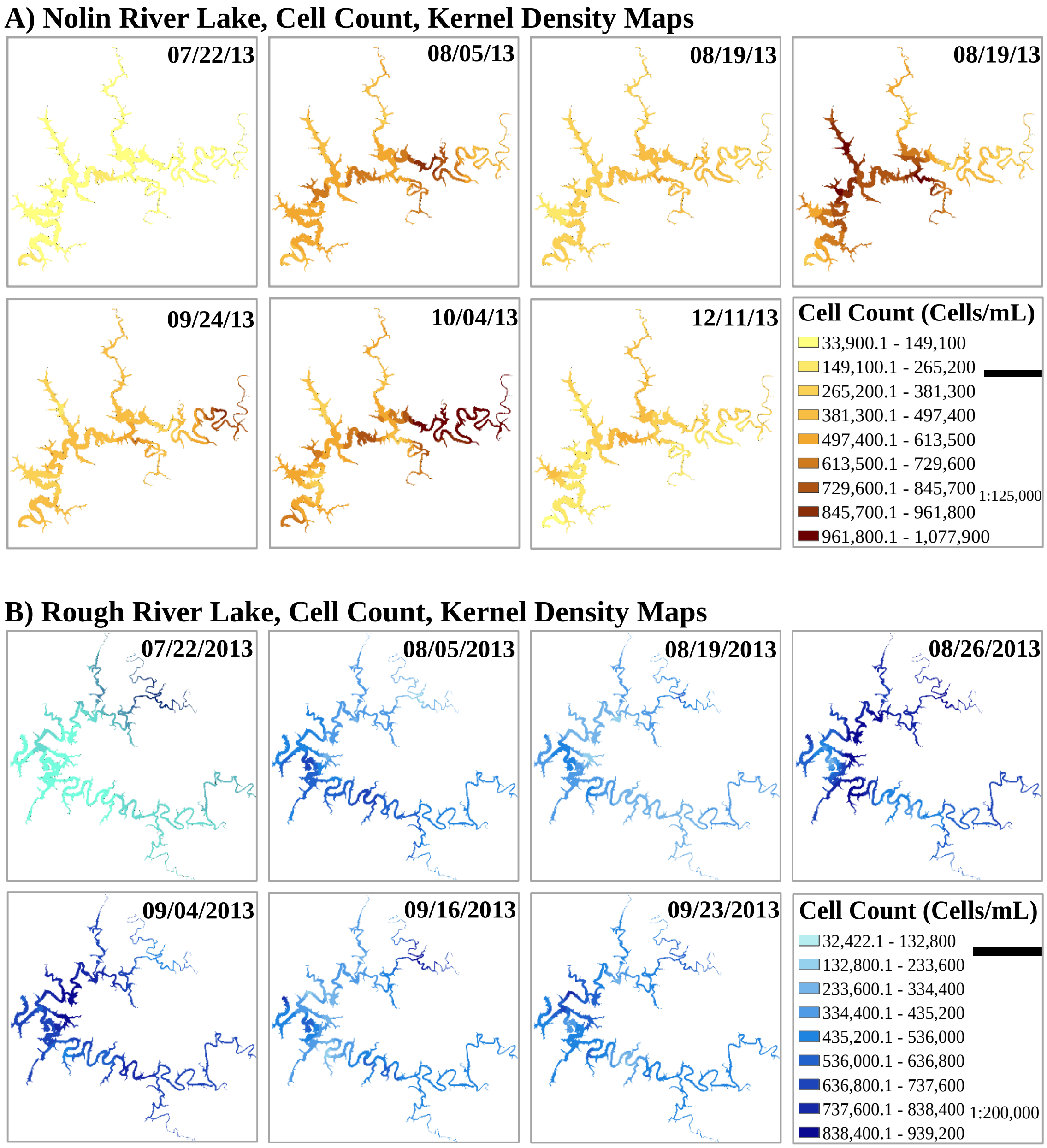



Figure 3.8. Nolin River Lake (A) and Rough River Lake (B) KDE maps. The USACE survey started on 7/22/13 (Julian Date 203) and ended 12/11/13 (Julian Date 345).. Here, the survey started on 7/08/13 (Julian Date 189, not shown) and ended on 9/23/13 (Julian Date 266). Both sets are of the kernel density estimations of the cell counts at each lake's USACE-established HAB sample sites. Not all sites were sampled each time, nor was either survey done on the same timescale and resolution. There is not a discernable temporal pattern for the distribution of the HABs; however, there were a few places throughout the lakes that had consistently higher cell counts than others. Notice how the cell counts remain high at Nolin River Lake even into December. There was a similar trend in the 2014 data but, due to the incomplete nature of that survey (no one site saw more than one sample period and one of the lakes only had a couple sample periods to represent the whole bloom season), only the 2013 data are shown here. Source: Created by the author from historic USACE data; USGS (2013b).

Potential sampling locations were then selected using these KDE maps, a landuse/land cover map for the two watersheds for Nolin and Rough River Lakes, and a map of the karst hydrologic inputs (these were derived from geologic maps and existing core data from the area). Once potential inputs were identified, three representative sites for karst inputs, along with the dam's tailwaters, were selected at each lake through evaluation of the GIS data, study site field visits, and guidance from the USACE (Figure 3.4). For Nolin River Lake these sites included the tailwaters (Site 1) for the lake's discharge; Pine Springs (Site 2) to catch the main signal from Nolin River; Iberia Recreation Area (Site 3) for its abundant springs and adjacent blue hole; and Conoloway (Site 4) for the local springs and historic "hotspots" of HAB activity as seen in the KDE maps (Figure 3.4 and Figure 3.8a). Rough River Lake sites also included the dam's tailwaters (Site 1); the Laurel Branch Campground (Site 2) as it has several large blue holes in close proximity to the sample site; Forest Lake (Site 3) for the local springs draining into the lake throughout Rough River North Fork; and Peter Cave (Site 4) for the signal from Rough River's main branch and the contribution from the sample site's namesake (Figure 3.4). All of the Rough River Lake sites also had repeated incidences of high cell counts according to the historic data (Figure 3.8b).

Bi-weekly sampling occurred at the eight selected sites from August 2015 to March 2016. At each site, a YSI 556 multiparameter handheld data collection device was used for grab samples to measure the geochemistry of the water. This device is equipped with probes to measure conductivity/temperature, pH, salinity, and dissolved oxygen; however, due to instrument error, DO values were not collected until the fall/seasonal transitional period into winter. Water samples for a variety of parameters (*E. coli*, cations, anions, and $NO_3$ isotopes) were also collected at each site. The sampling procedures varied at each site, but largely consisted of collecting water from the surface, or just below it, of the lakes and tailwaters. All the samples were collected by hand (using nitrile gloves), with the exception of one site. Rough River Lake's Site 3 was located on a bridge, so a bailer was used for sample collection.

Samples for *E. coli*, were collected using a 125 mL screw-top plastic sampling bottle with the preservative sodium thiosulfate ($Na_2S_2O_3$) per EPA Method 1603. The rest of the water samples were filtered through a 0.45µm pore filter. Cation samples were preserved with nitric acid in 30 mL Nalgene bottle. For the anion and isotope samples, the water was filtered a second time using a 0.2µm pore filter. Filtering the samples through these membranes eliminates contaminants from the sample and helps prevent denitrification and fractionation by the bacteria naturally present in water as well as removing all the non-dissolved species from the water. These samples were stored at $4^oC$ in the dark, until the samples could be delivered to the laboratory for analysis or, in the case of the isotopes samples, frozen. Freezing isotope samples further protected them from additional denitrification by inhibiting the respiration of any possible bacteria present in the sample.

The *E. coli* presences and concentrations were obtained from the water samples by analysis in WKU's HydroAnalytical Lab. A suite of anion (chloride, nitrates, phosphates, and sulfates) and cations (calcium, magnesium, iron, potassium, sodium, etc.) concentrations was obtained at the WKU Advanced Materials Institute. Additionally, water samples collected for a dual isotopic tracing of nitrate ($\delta^{18}O-NO_3$ and $\delta^{15}N-NO_3$) to determine the sources of the nutrient contaminates were analyzed at the

University of Maryland's Central Appalachian Stable Isotope Facility (CASIF). After they reached CASIF, they were further processed through the denitrifier method into nitrous oxide ($N_2O$) before being analyzed with mass spectronomy (Sigman et al. 2001; Casciotti et al. 2002; Kendall et al. 2007; Granger and Sigman 2009). Tertiary data, such as precipitation and discharge, were acquired with use of available weather stations (USACE, NOAA's CoCoRaHS online database) and the USACE discharge measurements for each dam (see Figure 3.6 and Figure 3.7).

The tertiary data, specifically discharge, were used to calculate the loadings of the nutrients of the lakes through Equation 3.1:

$$\text{2-Week Loading} = \text{Nutrient Con. (mg/L)} \times Q \text{ (L/sec)} \times 1{,}209{,}600 \text{ seconds} \qquad (3.1)$$

This equation calculates loading by first multiplying the two-week average discharge for the each sample period by the respective nutrient concentrations recorded during the concurrent time period. The product was then multiplied further by 1,209,600 seconds, or the number of seconds, in two weeks. This calculation operates under the assumption that each of these concentrations and resulting loadings were representative of the every other week sampling periods. The resulting products were then added together to get a total amount of the target nutrient that moved through the lake during the duration of the experiment. This number is only an approximation and its primary purpose is to get a better sense of the scale of the nutrient pollution.

### 3.4 Results/Discussion

Many factors are in play when it comes to CyanoHAB formation. Examining each of these factors in turn helps to determine the best method for better understanding conditions that can lead to HAB occurrence in karst landscapes. Over the course of eight months, data were collected at both Rough River Lake and Nolin River Lake. The resulting data from this study revealed interesting trends and provided some clues as to the cause of the lakes' winter bloom phenomenon, as well as the other seasonal occurrences and influences throughout the year.

During the course of this study only one CyanoHAB was documented at either lake. This bloom took place on Julian Date 260 (September 17, 2015) at Nolin River Lake (Figure 3.2). The bloom only lasted a few days and no sampling was done in or around it, due to communication errors. This survey only captured the conditions three days prior to the bloom (Julian Date 257, September 14, 2015) and eleven days preceding the event (Julian Date 271, September 28, 2015). Prior to the bloom and sampling day, a rain event moved through the drainage basin, as seen in the precipitation data in Figure 3.10. This event likely played a role in the formation of the bloom. A decrease in the nearly all anion and cation data was captured in response to this event. The decrease was likely due to a dilution effect of the freshwater recharge entering the lake and the typical rebound in these values is seen in the following sampling event. Unfortunately, due to the lack of data for the bloom itself little is known as to the cause of this event but, with the influx of groundwater, it is possible that additional nutrients and contaminants also entered the system. It should be noted that it is likely this was not the only CyanoHAB event to occur for either lake during the study; yet, no other CyanoHABs were documented.

Using historic cell-count data from previous USACE sampling, the primarily occurring cyanobacteria were identified. The data from HAB monitoring of the 2013 blooms indicate that cyanobacteria dominated the counts. At both Rough River Lake and Nolin River Lake, *Cylindrospermopsis raciborskii*, *Aphanocapsa*, *Pseudanabaena*, and *Chroococcus* were found to be the dominant cyanobacteria. *Romeria* also occurred at Rough River Lake in high numbers. All of these cyanobacteria have the capacity to produce toxins. *Cylindrospermopsis raciborskii* dominated the counts for most of the months, including the winter months at Nolin River Lake. This cyanobacteria is notable for being a nuisance species across the globe and has been documented producing toxins in several places; however, their capability to do so in North American strains is disputed (Jones and Sauter 2005; Yilmaz and Philips 2011). Regardless of the toxicity potential, *Cylindrospermopsis raciborskis* blooms still pose a threat to the water quality, ecosystem stability, and recreational use of the two lakes. With this target species in mind, the modern data were then inspected to determine how they relate to the bloom occurrences.

With four sites at each lake, it is practical to examine each set of sites in relation to their own lake, as no two sites are the same. In addition to this, seasons were defined for both lakes for consistency. The breakdown in the seasonal dates is based off the lakes' artificially controlled winter and summer pool elevations. Data collected from August 2015 until the beginning of drawdown, October 15, 2015, are considered to be collected during the summer pool stage. Data collected after November 2015 until the end of the study are considered to be part of the winter pool stage. The time in between is considered to be the transitional time period in which the lakes were being drawn down from summer pool to winter pool.

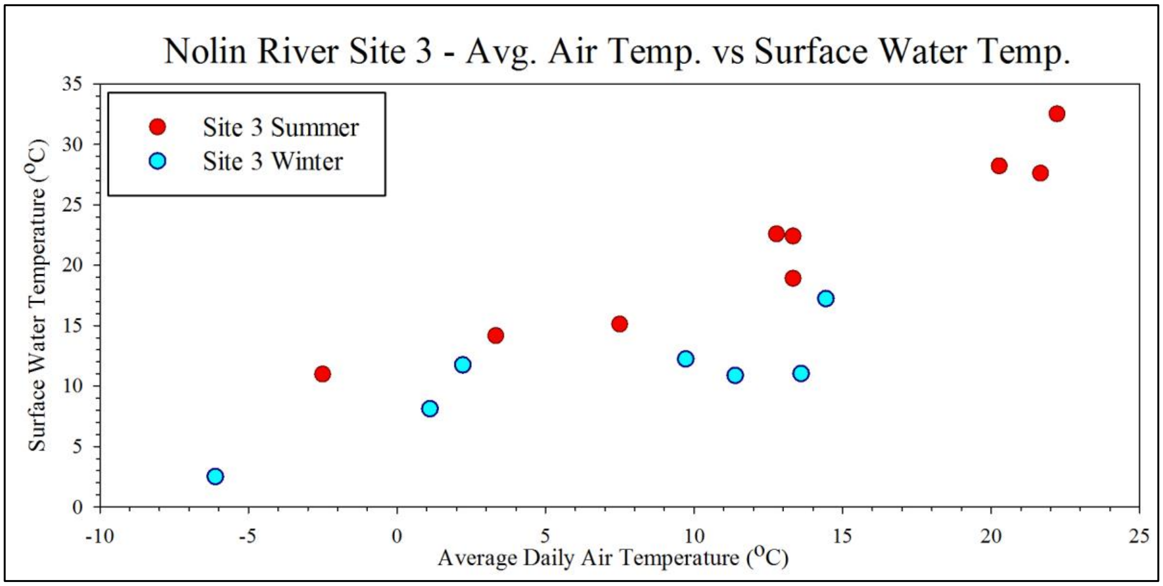



Figure 3.9. Nolin River Site 3 – Average Air vs Surface Water Temperature. When the average air temperature was plotted against the surface water temperatures for Nolin River Lake's Site 3, the winter temperatures showed the greatest variation, discounting the tailwaters, of all of the Nolin River Lake sites with a $R^2$ of 0.55 (p= <0.05) for the winter months (Aug-Oct '15). The summer month water temperatures saw a greater influence from the surface temperature with a $R^2$ of 0.86 (p= <0.05) Source: created by the author.

**Karst Groundwater Influences:** Nolin River Lake's main site of interest when looking for the influence of karst groundwater was the Iberia Recreation Area (Site 3). Nolin River Site 3 showed the strongest indication of a direct influence of karst hydrology within the temperature data. When the average daily temperatures were plotted against the surface water temperature, Site 3 had greater variation in the surface water temperature from the average air temperature during the winter months, with a $R^2$ value of 0.55 (p= <0.05) (Figure 3.9). This exhibits the controlling effects karst can have on surface water temperatures. The temperature of the water coming from karst sources tends to be the annual average temperature for that given location and is consistent, despite seasonal and precipitation influences (Palmer 2007). In the case of this region, the temperature of the groundwater tends to range between 12-14 °C year-round (Hess and White 1993). It is important to note that the average winter water temperatures of the surface water also stay near this range and are consistently higher than the daily average air temperatures at all of the sites within the lake (highlighted in Table 3.1) The basic water parameters reveal the seasonal change, as the surface water temperatures at Site 3 did not trend with average air temperatures (Figure 3.9, see Table 3.1 and Table 3.2 for specific numbers).

At all of the other sites, the surface water temperature remained consistently warmer than the average air temperature during the winter months (highlighted in Table 3.1 and Table 3.2). This trend is to be expected of surface waters, but the steady warmer nature of the water during the winter months could be from the karst influence. In this scenario, the warmer groundwater kept the surface water warm, despite the drop in the air temperature. These temperatures are low enough to inhibit other algal growth, but not cyanobacterial growth. Although the optimal temperature for *Cylindrospermopsis raciborskii* growth is 25-30 °C, it has been recorded growing in temperatures as low as 15 °C (Saker and Griffith 2001; Briand et al. 2004; Hamilton et al. 2005). The temperature differences were not the only karst groundwater indicators; additional evidence was detected at all of the sites in the pH variability. The pH of karst groundwater is normally high (above 7) and, throughout the study, the pH never dropped below 7.01 and got as high as 8.47 at the sites within Nolin River Lake (highlighted in Table 3.1). *Cylindrospermopsis raciborskii* complicates this matter, as past studies indicate that waters with higher pH and temperatures give cyanobacteria a competitive edge over green algal species (Yamamoto and Nakahara 2007). Furthermore, the cation data reveal groundwater influences as seen in the calcium and magnesium concentrations.

| Table 3.1: Descriptive Statistics Nolin River Lake | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Location | Site 1 - Tailwaters | | Site 2 - Pine Springs | | Site 3 - Iberia | | Site 4 - Conoloway | |
| Analyte | Statistic | Summer | Winter | Summer | Winter | Summer | Winter | Summer | Winter |
| Temp. ($^{o}$C) | Range | 17.10-25.50 | 4.07-13.62 | 20.60-33.10 | 1.80-16.62 | 22.40-32.50 | 2.50-17.22 | 22.47-35.40 | 11.56-18.30 |
| | Avg. | 22.29 | 9.80 | 25.57 | 10.39 | 26.65 | 10.98 | 28.83 | 14.60 |
| | Var. | 11.95 | 10.02 | 24.98 | 17.48 | 18.06 | 18.76 | 23.79 | 4.63 |
| pH | Range | 6.74-7.60 | 6.17-7.48 | 7.34-8.81 | 7.33-8.01 | 7.08-8.68 | 7.23-8.07 | 7.43-8.77 | 7.35-8.88 |
| | Avg. | 7.14 | 7.10 | 8.00 | 7.63 | 8.03 | 7.66 | 7.96 | 7.97 |
| | Var. | 0.09 | 0.24 | 0.48 | 0.04 | 0.38 | 0.07 | 0.30 | 0.25 |
| SpC (µS/cm) | Range | 274.20-645.00 | 178.00-245.00 | 216.50-501.00 | 204.00-385.00 | 201.00-266.80 | 74.00-188.00 | 191.80-276.40 | 127.00-533.00 |
| | Avg. | 378.02 | 218.63 | 279.80 | 288.25 | 233.56 | 122.38 | 236.14 | 233.29 |
| | Var. | 22838.36 | 443.70 | 15364.58 | 2859.36 | 659.51 | 1204.27 | 1371.49 | 19193.24 |
| DO (mg/L) | Range | n/a | 10.80-14.87 | n/a | 9.06-13.36 | n/a | 8.31-13.55 | n/a | 9.58-13.91 |
| | Avg. | n/a | 13.25 | n/a | 11.24 | n/a | 11.16 | n/a | 11.31 |
| | Var. | n/a | 2.39 | n/a | 2.53 | n/a | 3.11 | n/a | 2.21 |
| DO (%) | Range | n/a | 104.20-138.20 | n/a | 92.10-113.90 | n/a | 86.40-12.30 | n/a | 97.50-168.60 |
| | Avg. | n/a | 116.59 | n/a | 99.00 | n/a | 100.43 | n/a | 118.39 |
| | Var. | n/a | 151.90 | n/a | 77.74 | n/a | 117.45 | n/a | 645.71 |
| Sal | Range | 0.13-0.16 | 0.11-0.16 | 0.10-0.24 | 0.17-0.21 | 0.08-0.13 | 0.05-0.17 | 0.09-0.13 | 0.08-0.31 |
| | Avg. | 0.15 | 0.14 | 0.13 | 0.18 | 0.11 | 0.09 | 0.11 | 0.14 |
| | Var. | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| *E.coli* (MPN/100mL) | Range | 0.00-19.00 | 8.00-158.00 | 0.00-4.00 | 14.00-1153.00 | 0.00-16.00 | 16.00-1986.00 | 1.00-9.00 | 29.00-435.00 |
| | Avg. | 4.33 | 42.54 | 2.00 | 228.38 | 3.50 | 416.50 | 3.33 | 195.43 |
| | Var. | 55.07 | 2724.11 | 3.20 | 142540.84 | 37.90 | 442749.43 | 10.27 | 27509.62 |
| $NO_3$ (ppm) | Range | 0.24-4.48 | 1.45-8.19 | 0.00-1.13 | 8.41-12.91 | 0.00-0.27 | 0.32-1.99 | 0.00-0.19 | 0.23-3.75 |
| | Avg. | 2.57 | 6.34 | 0.27 | 11.19 | 0.15 | 0.95 | 0.14 | 1.71 |
| | Var. | 2.35 | 5.37 | 0.18 | 1.83 | 0.01 | 0.25 | < 0.01 | 1.31 |
| Ca (ppm) | Range | 24.14-46.63 | 28.22-43.46 | 20.59-31.75 | 36.56-54.43 | 25.20-35.12 | 11.78-30.72 | 25.72-40.97 | 19.01-42.40 |
| | Avg. | 38.42 | 37.16 | 25.45 | 48.94 | 30.31 | 17.59 | 32.15 | 27.97 |
| | Var. | 107.95 | 30.86 | 16.62 | 34.25 | 13.86 | 42.66 | 28.55 | 76.15 |
| Mg (ppm) | Range | 4.50-4.86 | 3.06-4.60 | 4.08-4.94 | 3.91-5.46 | 4.04-4.73 | 2.55-5.72 | 3.96-4.81 | 3.26-22.71 |
| | Avg. | 4.69 | 4.00 | 4.54 | 4.73 | 4.30 | 3.72 | 4.23 | 7.79 |
| | Var. | 0.02 | 0.21 | 0.09 | 0.25 | 0.08 | 1.07 | 0.10 | 47.91 |

Table 3.1. Descriptive statistics for the study data collected at Nolin River Lake. The average winter temps are highlighted to illustrate how they are closer to the average groundwater temp for the area (12-14 °C) than the average winter air temp (6 °C). The average pH values are highlighted to show how they remained above 7, regardless of season. The highlighted *E. coli* counts illustrate the large amount of waste entering the lake during the winter months. The highlighted winter $NO_3$ concentrations were highlighted to show the discrepancy between the main tributary of Nolin River Lake (Nolin River, as represented by Nolin Site 2) and the tailwaters (Nolin Site 1). Source: created by author.

Table 3.2. Descriptive statistics for the study data collected at Rough River Lake. The average winter temps are highlighted to illustrate how they are closer to the average groundwater temp for the area (12-14 °C) than the average winter air temp (7 °C). The average pH values are highlighted to show how they remained above 7, regardless of season. The highlighted *E. coli* counts illustrate the large amount of waste entering the lake during the winter months. The highlighted winter $NO_3$ concentrations show the discrepancy between the main tributary of Rough River Lake (Nolin River, as represented by Rough Site 4) and the tailwaters (Rough Site 1). Source: created by the author.

**Table 3.2: Descriptive Statistics Rough River Lake**

| | Location | Site 1 - Tailwaters | | Site 2 - Laural Branch | | Site 3 - Lake Forest | | Site 4 - Peter Cave | |
|---|---|---|---|---|---|---|---|---|---|
| Analyte | Statistic | Summer | Winter | Summer | Winter | Summer | Winter | Summer | Winter |
| Temp. ($^{o}$C) | Range | 23.94-32.60 | 8.88-16.25 | 24.45-38.10 | 3.92-19.35 | 17.50-30.20 | 2.90-19.37 | 24.38-37.20 | 5.39-17.17 |
| | Avg. | 28.38 | 11.46 | 29.43 | 12.68 | 25.49 | 12.22 | 29.02 | 11.11 |
| | Var. | 10.73 | 7.12 | 27.36 | 26.03 | 22.88 | 26.12 | 26.93 | 14.83 |
| pH | Range | 6.77-7.47 | 7.24-7.94 | 7.82-7.29 | 7.23-8.01 | 7.68-8.43 | 7.38-8.36 | 7.89-8.89 | 7.52-7.74 |
| | Avg. | 7.20 | 7.58 | 7.82 | 7.60 | 8.06 | 7.70 | 8.47 | 7.74 |
| | Var. | 0.07 | 0.06 | 0.20 | 0.07 | 0.12 | 0.12 | 0.19 | 0.03 |
| SpC (µS/cm) | Range | 220.00-267.40 | 148.00-224.00 | 199.60-244.10 | 99.00-286.00 | 194.90-232.20 | 103.00-286.00 | 212.10-239.50 | 177.00-29.00 |
| | Avg. | 243.74 | 189.29 | 215.48 | 179.50 | 209.36 | 200.63 | 225.94 | 234.00 |
| | Var. | 413.73 | 861.24 | 437.04 | 5158.57 | 189.67 | 4885.41 | 117.81 | 1931.43 |
| DO (mg/L) | Range | n/a | 9.70-14.33 | n/a | 7.52-13.29 | n/a | 8.21-14.49 | n/a | 8.08-17.15 |
| | Avg. | n/a | 11.66 | n/a | 9.62 | n/a | 10.59 | n/a | 11.69 |
| | Var. | n/a | 3.82 | n/a | 4.34 | n/a | 4.20 | n/a | 8.30 |
| DO (%) | Range | n/a | 96.90-126.20 | n/a | 74.00-110.20 | n/a | 82.50-107.50 | n/a | 84.10-135.60 |
| | Avg. | n/a | 107.64 | n/a | 90.39 | n/a | 98.46 | n/a | 105.13 |
| | Var. | n/a | 148.13 | n/a | 155.71 | n/a | 81.75 | n/a | 266.55 |
| Sal | Range | 0.10-0.13 | 0.08-0.15 | 0.09-0.11 | 0.07-0.18 | 0.09-0.11 | 0.07-0.17 | 0.10-0.11 | 0.12-0.18 |
| | Avg. | 0.12 | 0.11 | 0.10 | 0.11 | 0.10 | 0.13 | 0.10 | 0.15 |
| | Var. | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| *E. coli* (MPN/100mL) | Range | 4.00-50.00 | 6.00-365.00 | 1.00-81.00 | 11.00-2420.00 | 0.00-16.00 | 28.00-1986.00 | 0.00-8.00 | 54.00-2420.00 |
| | Avg. | 20.17 | 83.41 | 23.50 | 474.86 | 3.00 | 375.50 | 2.83 | 494.25 |
| | Var. | 331.37 | 16184.73 | 989.90 | 773988.81 | 40.80 | 433686.86 | 8.17 | 669034.21 |
| $NO_3$ (ppm) | Range | 0.09-0.70 | 2.19-4.20 | 0.06-0.22 | 1.73-3.18 | 0.11-0.21 | 1.69-3.75 | 0.08-0.23 | 3.60-5.19 |
| | Avg. | 0.42 | 2.99 | 0.13 | 2.18 | 0.14 | 2.75 | 0.14 | 4.35 |
| | Var. | 0.04 | 0.69 | < 0.01 | 0.26 | < 0.01 | 0.40 | < 0.01 | 0.23 |
| Ca (ppm) | Range | 30.44-39.50 | 22.78-40.14 | 29.62-35.35 | 18.29-42.94 | 19.09-30.83 | 18.39-46.96 | 12.22-37.31 | 34.76-51.01 |
| | Avg. | 36.41 | 31.94 | 32.01 | 30.34 | 26.68 | 37.10 | 28.49 | 45.08 |
| | Var. | 13.53 | 50.77 | 3.82 | 98.16 | 29.49 | 110.39 | 149.68 | 40.09 |
| Mg (ppm) | Range | 3.56-3.99 | 2.75-4.21 | 3.00-3.61 | 2.05-5.02 | 2.91-3.34 | 2.16-4.55 | 3.42-4.19 | 3.51-5.60 |
| | Avg. | 3.79 | 3.51 | 3.32 | 3.48 | 3.13 | 3.63 | 3.81 | 4.45 |
| | Var. | 0.03 | 0.27 | 0.04 | 1.16 | 0.03 | 0.74 | 0.08 | 0.51 |

At Nolin River Lake, concentrations of calcium steadily increased at most of the sites with the progression of winter, with few exceptions (Figure 3.10). At each site, the calcium concentrations increased by nearly double, with Site 2 (Pine Springs) having the largest seasonal change (Table 3.1, Figures 3.10 and 3.11). These dramatic increases in calcium during the winter could be indicative of increases in karst groundwater inputs, as the calcium concentration in karst groundwater is typically higher than that of surface waters and longer residence times in winter can increase the amount of carbonate dissolution. The magnesium concentrations increased from 4.37 ppm in the summer to 4.96 ppm in the winter. As the lake level receded to winter pool, the influence of the karst groundwater seemed to increase. More evidence is presented when examining the relationships between calcium and magnesium (Figure 3.11).

Examining the relationships between calcium, magnesium, and SpC can help delineate surface water from groundwater (Rugel et al. 2016). In examining these relationships for Nolin River Lake's sites, Site 3 appears to have the greatest karst influence, with a $R^2$ of 0.87 ($p = <0.05$) for calcium versus SpC values, indicating that calcium makes up nearly 87% of the dissolved ions in the water for the duration of the study period. This interpretation is also supported by the karst water pulse seen in Figure 3.11 around Julian Date 327 (November 21, 2015). The close relationship between calcium and SpC does not appear in the tailwaters, which is likely due to the larger amount of mixing occurring at the dam before the water is discharged. It should be noted that this relationship between SpC and calcium only means that the majority of the dissolved ions in the water were made up of calcium; while this is indicative of carbonate

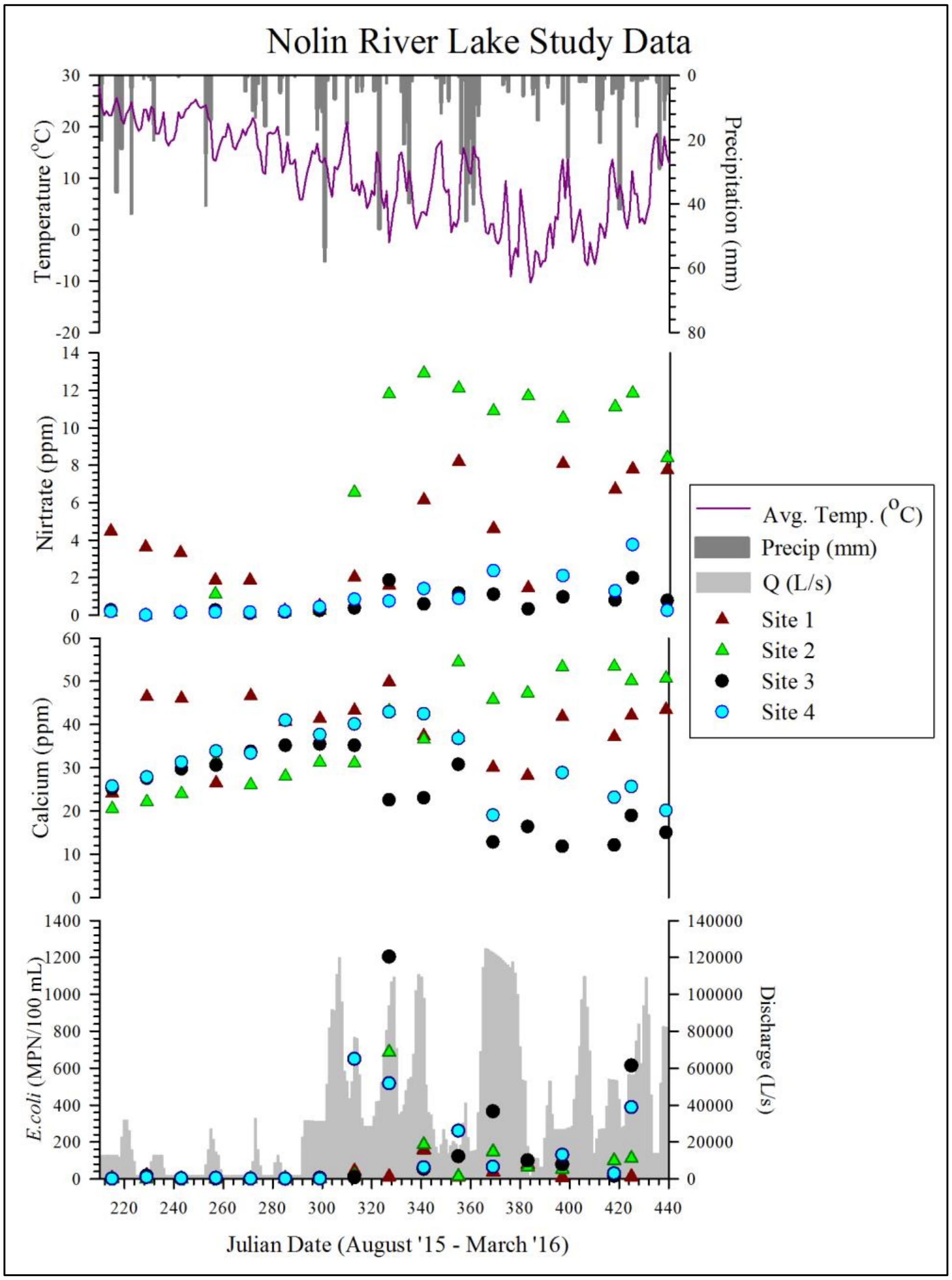
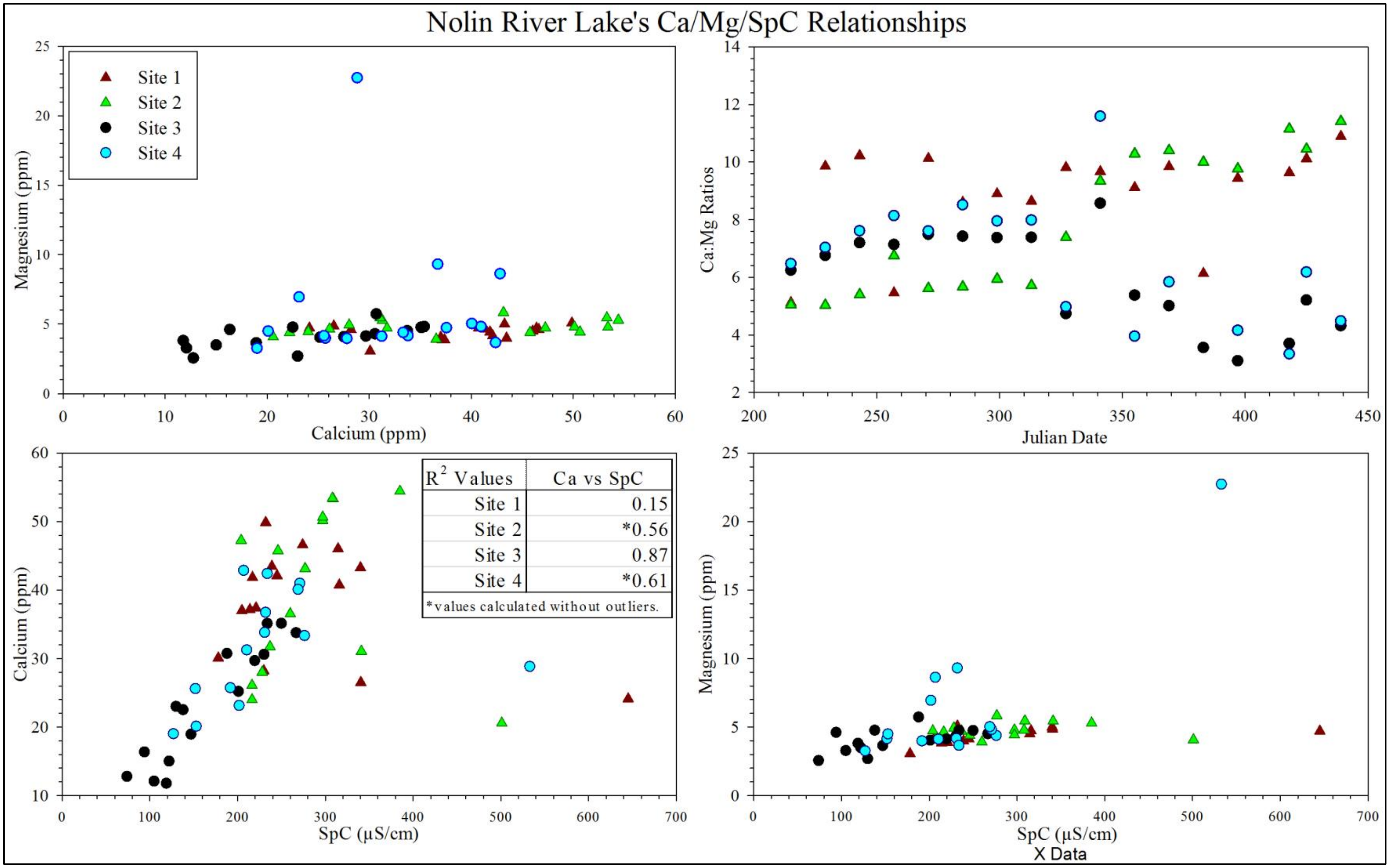



Figure 3.10. Nolin River Lake Study Data. Weather, $NO_3$, $Ca^+$, and *E. coli* concentrations for Nolin River Lake sites over the duration of the study. Note the overall increase in parameters with the onset of winter. Possible groundwater pulses can be seen on Julian Dates 327 and 425, November 21st and February 29th, respectively. Source: created by the author.




| $R^2$ Values | Ca vs SpC |
|---|---|
| Site 1 | 0.15 |
| Site 2 | *0.56 |
| Site 3 | 0.87 |
| Site 4 | *0.61 |

*values calculated without outliers.

Figure 3.11. The relationships between SpC, Ca, and Mg. The higher $R^2$ for Site 3's Ca vs SpC is indicative of karst groundwater. It can be inferred that the elevated SpC values are accounted for by the elevated Ca values. Naturally occurring karst waters are dominated by Ca (all p=<0.05). Source: created by author.

groundwater water, other relationships must also be explored (Rugel et al. 2016). This is the case for Site 3's Ca:Mg ratios, these ratios are indicative of south-central Kentucky's karst aquifer (Shuster and White 1971; Hess and White 1993). They indicate that the water contains dissolved limestone and the seasonality of the data mirrors that of other studies of the geochemistry of the karst springs on the nearby Green River (Hess and White 1993). After Julian Date 344 (November 30, 2015) Nolin River Lake Site 1 and 2's Ca:Mg ratios track with each other, with the average Ca:Mg ratio of 9.35 and 10.35 for Site 1 and Site 2, respectively, while the rest of the sites varied greatly. Since Nolin River Site 2 is located on the main tributary of the lake, Nolin River, it is likely the leading contributor of water during the winter base flow and this conclusion is supported in the trend seen there. The only time the sites do not trend together was on Julian Date 383 (January 18, 2016) (Figure 3.11). This discrepancy can also be observed in the other data, indicating a hydrological phenomenon took place that was likely related to the spike in discharge prior to the sample period (Figure 3.10). The Ca:Mg ratios are still indicative of karst groundwater influence, however, with the ratios never falling below 3.3; this indicates the consistent karst influence in the surface water.

Rough River Lake did not have any one site with a greater karst groundwater influence in respect to temperature, as none of the sites within the lake showed a significant difference in temperatures like Site 3 at Nolin River Lake; however, the water temperatures remained well above that of the average air temperature and above the minimum temperatures needed for cyanobacteria to flourish with little competition. The pH at sites within Rough River Lake remained high throughout the year, never dropping below 7; thus, also providing a competitive edge to the cyanobacteria (highlighted in

Table 3.2) (Yamamoto and Nakahara 2007). The elevated pH could be from karst groundwater inputs and this conclusion is supported by the cation data (Figure 3.10 and 3.12). The cation data for Rough River Lake indicate elevated levels of calcium and magnesium that increased with the onset of winter. The concentrations of calcium increased from 28.49 ppm in the summer to 45.08 ppm in the winter for Site 4, marking the most dramatic of the changes (Table 3.2, Figure 3. 12). This could indicate a longer residence time and likely less dilution from summer storm events.

The SpC data should, in turn, have increased with the increasing concentrations of the ions; however, for Rough River Lake, the average SpC values overall decreased from 224.48 µS/cm at summer pool average to 203.14 µS/cm at winter pool (Table 3.2). While this sort of decrease would indicate a reduction in dissolution, or the karst groundwater input influence, the decrease also coincided with an increase in discharge. This coincidence, coupled with the lower water levels and decreased residency time of the lake water, could indicate that karst waters have a stronger influence in winter as this flow regime creates an environment of higher mixing rates and lower chances for thermal stratification. The calcium and magnesium anions, indicators of carbonate rock dissolution, increased as the winter progressed, further demonstrating that, despite the decrease in SpC values, there was still karst groundwater entering the lakes (Figure 3.12). The only lake site that showed the typical relationship between calcium and SpC in a karst system was Site 2, with a $R^2$ value of 0.6 ($p= <0.05$) (Figure 3.13). This correlation could indicate that the water moving through the system is coming from carbonate-rich groundwater sources. The ratios track with the increase and decrease in water, the

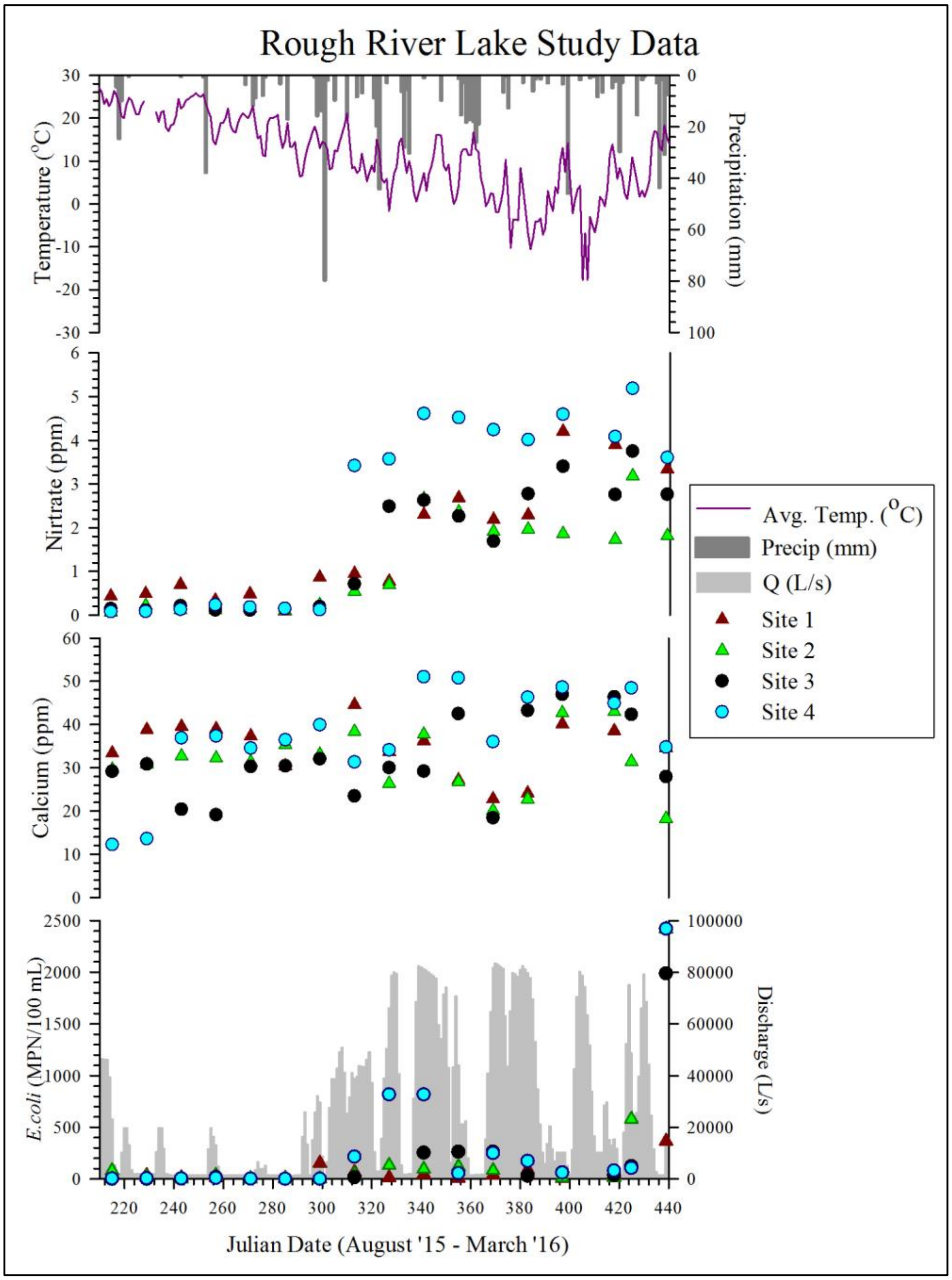



Figure 3.12. Rough River Lake Study Data. Weather, $NO_3$, $Ca^+$, and *E. coli* concentrations for Rough River Lake sites over the duration of the study. Note the overall increase in parameters with the onset of winter, although the change in signal is weaker than that of Nolin River Lake. Also, note how storm influences seem to be stronger in the winter with the spikes in the data during November - December 2015. Source: created by the author.

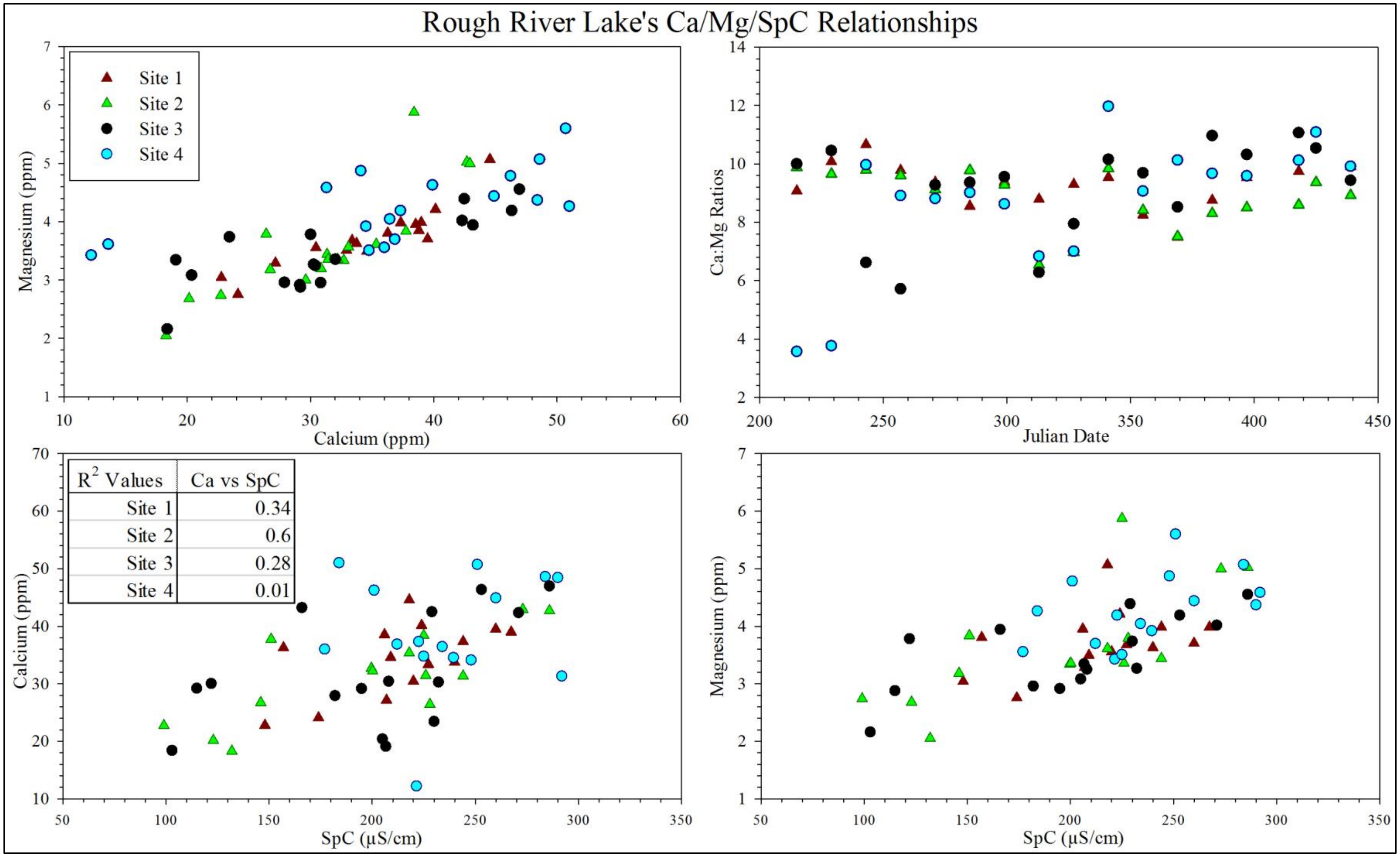



Figure 3.13. Rough River Lake Ca/Mg/SpC Relationships. As indicated by the $R^2$ value of 0.60, Site 2 had the strongest Ca vs SpC relationship, which is typically indicative of karst groundwater influence (all p= <0.05). Source: created by the author.

seasonality of the $CO_2$, and carbonate rock dissolution in a karst aquifer (Shuster and White 1971; Hess and White 1993). This evidence answers the question regarding the influence karst plays in the lake's recharge and some of the influence that karst groundwater plays in CyanoHAB formation; however, it does not address the nutrient issue.

**Nutrient Pollution**: Kentucky's karst landscape not only makes the water entering the lakes more suitable for cyanobacteria growth, but it also contributes large amounts of anthropogenic nutrient pollution, which contributes to HAB formation. Both lakes had large increases in nutrients concentrations during the winter months and some interesting patterns exist, especially with regard to the DO data. An overall increase in DO values occurred at each lake as winter progressed, likely due to a decrease in the productivity in the zooplankton with the lowering of the water temperature. *E. coli* cell counts also increased with the onset of winter, even increasing several orders of magnitude in some cases.

Nolin River Lake's cell counts increased from zero detectable *E. coli* to a maximum of 1,986 MPN/100 mL. Rough River Lake had a similar increase, with a higher maximum value of 2,420 MPN/100 mL (highlighted in Table 3.1 and 3.2). This increase coincided with a steep increase in the nitrates concentrations (Figure 3.10 and 3.12). The spike in *E. coli* was accompanied an increase in the calcium, SpC, and nitrate data, indicating that it was likely part of a karst groundwater pulse (Figure 3.10 and 3.12). These pulses typically occur in response to storm events and can originate from anywhere within the karst basin, thereby meaning that a storm event does not have to be local for a response to be seen in the geochemistry. Such a pulse of groundwater was detected in

Nolin River Lake Site 2's (Pine Springs) data around Julian Date 327 (November 21, 2015) (Figure 3.10). In fact, Site 2 had the highest nitrate concentration, 12.91 ppm $NO_3$, recorded for the duration of the study after another storm around Julian Date 341 (December 7, 2015) (Figure 3.10). Nolin River Lake Site 4 also experienced a spike in nitrate concentrations following the same storm event (Figure 3.10). Throughout Nolin River Lake's sample sites, the average concentration of $NO_3$ was 0.90 ppm, while the winter pool concentrations averaged around 5.14 ppm (Table 3.1).

Rough River Lake's sites had similar patterns. At most of the sites, karst groundwater influences were detected in the winter data. At this lake, $NO_3$ average values were 0.22 ppm for summer pool and 3.10 ppm for winter pool (Table 3.2). Phosphate concentrations were unsurprisingly low, only being detected once at Nolin River Lake's tailwaters (0.47 ppm) during the winter months and not at all at Rough River Lake. This is likely due to many factors, not limited to, but including, calcite's affinity for phosphorus, the limited availability of phosphate in freshwater ecosystems, and its subsequent rapid use/depletion. In all likelihood, any high concentrations of phosphorous were consumed before they were detected during the study, largely due to the sampling resolution. This explanation is likely, because some cyanobacteria species will store any phosphate that the bacteria come in contact and use it, regardless of availability, as is the case with *Cylindrospermopsis raciborskii* (Isvánovics et al. 2000); thus, further highlighting the importance of studying nitrate concentrations along with phosphate.

In both lakes, the *E. coli* concentrations, calcium concentrations, and SpC also trend together with the onset of winter pool and even the nitrate values sharply increased as the water levels fell. This was likely due to a combination effect of the sediments being disturbed by drawdown and the reduction in the amount of water in the lakes. As winter progressed, the temperatures decreased in the atmosphere and in the upper layers of the soil, thereby leading to a decrease in the production soil-based bacteria and top cover normally associated with reducing the nutrient pollution and filtering the water before it makes it into the epikarst (Williams 2008). The abundance of unprocessed and excess processed nitrates then get flushed through the study area's thin soils into the karst groundwater. Nutrient enriched groundwater then ends up in the lakes and rivers, a phenomenon that holds true for this study's dataset. The seasonal shift and the elevated cold tolerance of *Cylindrospermopsis raciborskii* are likely factors allowing the Nolin River Lake's winter blooms to occur.

| **Table 3.3**: Seasonal and Total $NO_3$ Loadings | | | | |
|---|---|---|---|---|
| Lake/Site | Tailwaters | | Tributaries | |
| Nolin Summer* | 120,000 | Kg | 38,000 | Kg |
| Nolin Winter** | 3,600,000 | Kg | 7,800,000 | Kg |
| Nolin Total | 4,000,000 | Kg | 9,700,000 | Kg |
| Rough Summer* | 20,000 | Kg | 18,000 | Kg |
| Rough Winter** | 980,000 | Kg | 4,200,000 | Kg |
| Rough Total | 1,100,000 | Kg | 4,700,000 | Kg |
| *Aug '15 - Oct '15; **Nov '15 - Mar '16; Loadings for Oct-Nov not shown. | | | | |

Table 3.3. The seasonal and total nitrate loadings for Nolin and Rough River lakes. Note the difference in the seasonal loading between each lake's tributaries and the output of the dam found in the tailwaters. Source: created by the author.

The nutrient pollution not only is impacting the lakes, but also the rivers downstream. Examining the nutrient data within the context of discharge highlights just how much nutrients have moved through the system during the course of the study, as well as some of the dynamics of nutrient pollution in the context of the lake. For Nolin River Lake, approximately 4,000,000 kg of nitrate discharged into the Green River during the course of this study, with approximately 9,700,000 kg of nitrate entering the lake through the tributaries represented by the sampling sites. At Rough River Lake, about 1,100,000 kg of nitrate was discharged through the dam, while roughly 4,700,000 kg of nitrate was detected coming through the represented tributaries.

This discrepancy in numbers can be further examined by breaking the loadings down seasonally (Table 3.3). At Nolin River Lake approximately 38,000 kg of nitrate entered through the tributaries and about 120,000 kg exited through the tailwaters during the summer months (August through October). This discrepancy could be due to uncaptured nitrate loading occurring from other tributaries on the lake, which could come from marinas, the recreational boaters, and leaking septic systems of the summer homes around the lake. It could also be due to a concentration effect at the dam, which was only seen in the tailwaters. The winter months (November 2015 through March 2016) saw a drastic increase in nitrate loadings, with roughly 7,800,000 kg of nitrate entering the lake through the represented tributaries and 3,600,000 kg of nitrate exiting through the tailwaters (Table 3.3).

The lack of expected nitrate coming through the dam is likely due to biological activity. During this time period, *E. coli* numbers increased in concentration and cyanobacterial numbers were high in the historic data during the same time seasonal period in the past. These organisms consume dissolved nitrate in great quantities when their concentrations are high and this could account for the drop in the dissolved nitrates coming through the dam. A case could also be made for the increase in discharge at the tailwater attributing for the lower nitrate concentrations. Similarly, Rough River Lake saw lower nitrate loadings during the summer, with approximately 18,000 kg of nitrate coming into the lake through the lake's sample sites and about 20,000 kg of nitrate leaving through the dam's tailwaters (Table 3.3). Unlike Nolin River Lake, Rough River Lake's concentrations pre- and post-dam are similar, which could be attributed to the nitrogen affixing nature of many of cyanobacterial species. In conditions of low nitrate availability, several cyanobacterial species, including *Cylindrospermopsis raciborskii*, have the capacity to affix nitrogen (in the form of $N_2$) out of the air (Paerl et al. 2001). There was a shift and increase in nitrate loading at Rough River Lake similar to the shift and winter loadings at Nolin River Lake with approximately 4,200,000 kg of nitrate entering through the tributaries and nearly 980,000 kg of nitrate exiting the lake in the tailwaters (Table 3.3). Again, this difference in loadings is likely due to either bioactivity or dilution in the discharge at the dam, or it could be a combination of the two effects. All of these amounts are high and considering that a large portion of the study was done in the winter, it lends even more credit to the evidence supporting the idea that eutrophication is aiding in the HAB formations on these lakes and is likely from the influence of karst groundwater. Given that the water moving through these riverine systems is karst in nature, the nutrient data can be further examined to determine the sources from which they originated prior to entering the groundwater system.

**Pollution Sources:** The isotopic data indicate one definitive result, which is the majority of nitrates entering the system are not entering through the atmospheric deposition of precipitation; however, they do suggest several possible non-point pollution sources. There is great variation among the individual sites at each lake and these sources often do not match the local land use; this adds more credibility to the explanation that the nutrients are being delivered to the system through karst groundwater inputs.

At Nolin River Lake, the overall trend in the isotope data seems to point towards a mix of inorganic, ammonium ($NH_4^+$)-based fertilizers and animal/septic waste sources, with an emphasis on the waste, during the summer months, with $\delta^{18}O$-$NO_3$ values ranging from +6.37‰ to +23.21‰ and $\delta^{15}N$-$NO_3$ ranging from +0.42‰ to +27.19‰ (Figure 3.13a). The winter month $\delta^{18}O$-$NO_3$ values ranged from -2.2‰ to +27.59‰ and +4.23‰ to +11.45‰ for the $\delta^{15}N$-$NO_3$ values, respectively, indicating the nitrates were mostly derived from natural soil $NH_4$/$NO_x$ sources, with some septic/animal waste added (Figure 3.13b). These sources are not exactly what are expected for the lake given the seasonal landuses. The clustering of the winter data also raises several questions, and discussing each site and its respective data separately helps to shed light on the dichotomy of the summer and winter data.

For Nolin River Lake's Site 1 (tailwaters), a majority of the nitrates are coming from animal/septic waste ($\delta^{15}N$ +10.43‰ to +27.19‰; $\delta^{18}O$ +6.37‰ to +23.21‰) during the summer months and a mixture of $NH_4^+$ fertilizers and soil $NH_4$/$NO_x$ during the winter months ($\delta^{15}N$ +5.19‰ to +6.62‰; $\delta^{18}O$ +1.43‰ to +8.49‰). There also appears to be some denitrification occurring during the summer months (August-October) indicated by the isotope values falling along the denitrification line in Figure 3.14a. This denitrification is the result of the biological processes enacted upon the nitrate by bacteria. Where and when the denitrification occurred is unknown. It could have occurred in the soil, aquifer, or the lake itself. The latter seems more likely, as during summer pool the lake tends to stratify as residency times increase, allowing bacteria and cyanobacteria to process any nitrates in the water before has a chance to exit through the tailwaters. This is a rapid process and difficult to capture in the isotopic data (Lehmann et al. 2004).

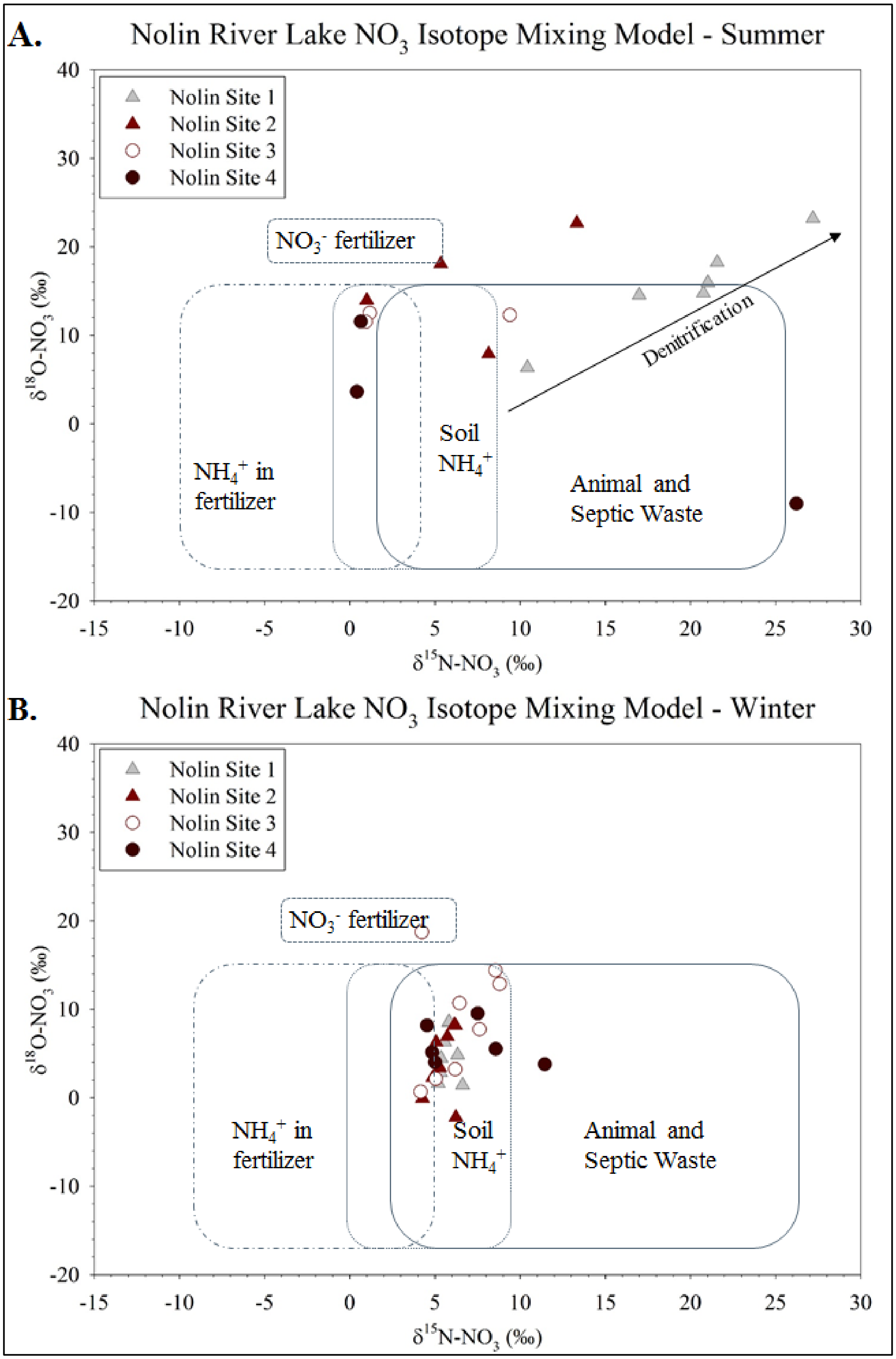



Figure 3.14. The mixing model for Nolin River Lake. A) Summer, and B) winter, $\delta^{18}O\text{-}NO_3$ and $\delta^{15}N\text{-}NO_3$ values. These dual isotope mixing models were modified from Kendall et al. (2007).
Source: created by author, with modification from Kendall et al. (2007).

Site 2 (Pine Springs and Nolin River) saw a mix of inorganic $NH_4^+$ fertilizers and organic soil $NH_4/NO_x$ year-round ($\delta^{15}N$ +1‰ to +13.33‰; $\delta^{18}O$ -2.2‰ to +22.69‰), with a focus on fertilizers for the duration of the summer pool period (Figure 3.14a). The majority of the local land use around this site is hay/pasture lands, but given the site also likely represents the signal from the main tributary, Nolin River, the watershed's sinkhole plain probably played a large role in nutrient pollution delivery and concentration (Figure 3.3). Since the land use around the sinkhole plain is largely agricultural, these data highlight the importance of focusing community outreach and education efforts on the people upstream of the lake, not just the local inhabitants. The nitrate isotope data from Site 3 (Iberia Recreation Area) indicate $NH_4^+$ fertilizers are the primary sources of nitrate during the summer ($\delta^{15}N$ +0.94‰ to +9.4‰; $\delta^{18}O$ +11.52‰ to +12.53‰) and then it switches solely to a mixture of septic/animal waste and organic soil $NH_4/NO_x$ as the primary source in the winter months ($\delta^{15}N$ +4.16‰ to +8.8‰; $\delta^{18}O$ +0.68‰ to +18.72‰). Interestingly, the local land cover for this recreation area is mostly forest. No major agriculture or urban development is adjacent to the sample site; however, a small lakeside community rests on the leeside of the ridge behind Site 3 and the sample site was fed by a collection of springs with a blue hole in the lake's main channel. Therefore, any nitrates entering the system are not likely to be from local sources, but derived from rapid flowing conduits farther out in the recharge area. This conclusion is supported by the winter isotopic data in that $\delta^{15}N$ values +2‰ to +5‰ tend to come from $NH_4/NO_x$ in cultivated soils, indicating an agricultural source (Kendall 1998). Again, this highlights the importance of having a broad education and community outreach program that includes everyone in the watershed, not just the local stakeholders. In this karst region, where contaminants can move vast distances over short time periods it is important to not just consider the immediate surroundings and landuses, but all the possible sources of nutrient overload, or in the case of Nolin River Lake, agricultural, recreational, urban, and natural land use.

The major nitrate contributors at Site 4 (Conoloway) are the same as Site 3, but with slightly more animal/septic waste input during the winter months (Figure 3.14b). This site is adjacent to a lakeside community, so a strong signal from septic waste contribution was expected, since all of the residences in the local area are on septic systems. Interestingly, this still does not account completely for the shift in the isotopic data from summer to winter. The data discrepancy is compounded when examining the trends found within the Rough River Lake isotope data.

The isotopic data from Rough River Lake showed some similar patterns, especially in relation to the summer to winter shift, but the data were ultimately different in mixtures and concentrations (Figure 3.15a-b). For Site 1 (tailwaters), the summer month nitrates were mix of animal/septic waste and $NH_4^+$ fertilizers ($\delta^{15}N$ -1.08‰ to +16.97‰; $\delta^{18}O$ -9.02‰ to +11.55‰) (Figure 3.15a). The isotope values shift in the winter months to change the mixture to that of animal/septic waste and natural soil $NH_4/NO_x$ (δ15N +4.71‰ to +5.73‰; δ18O +3.78‰ to +9.52‰) (Figure 3.15b). This site had the most variability in its isotopes, which can be explained by the tailwaters experiencing elevated amounts of mixing when compared to the rest of the lake, because the source of the tailwaters is human-controlled inputs from the dam, which varied regularly throughout the study period.

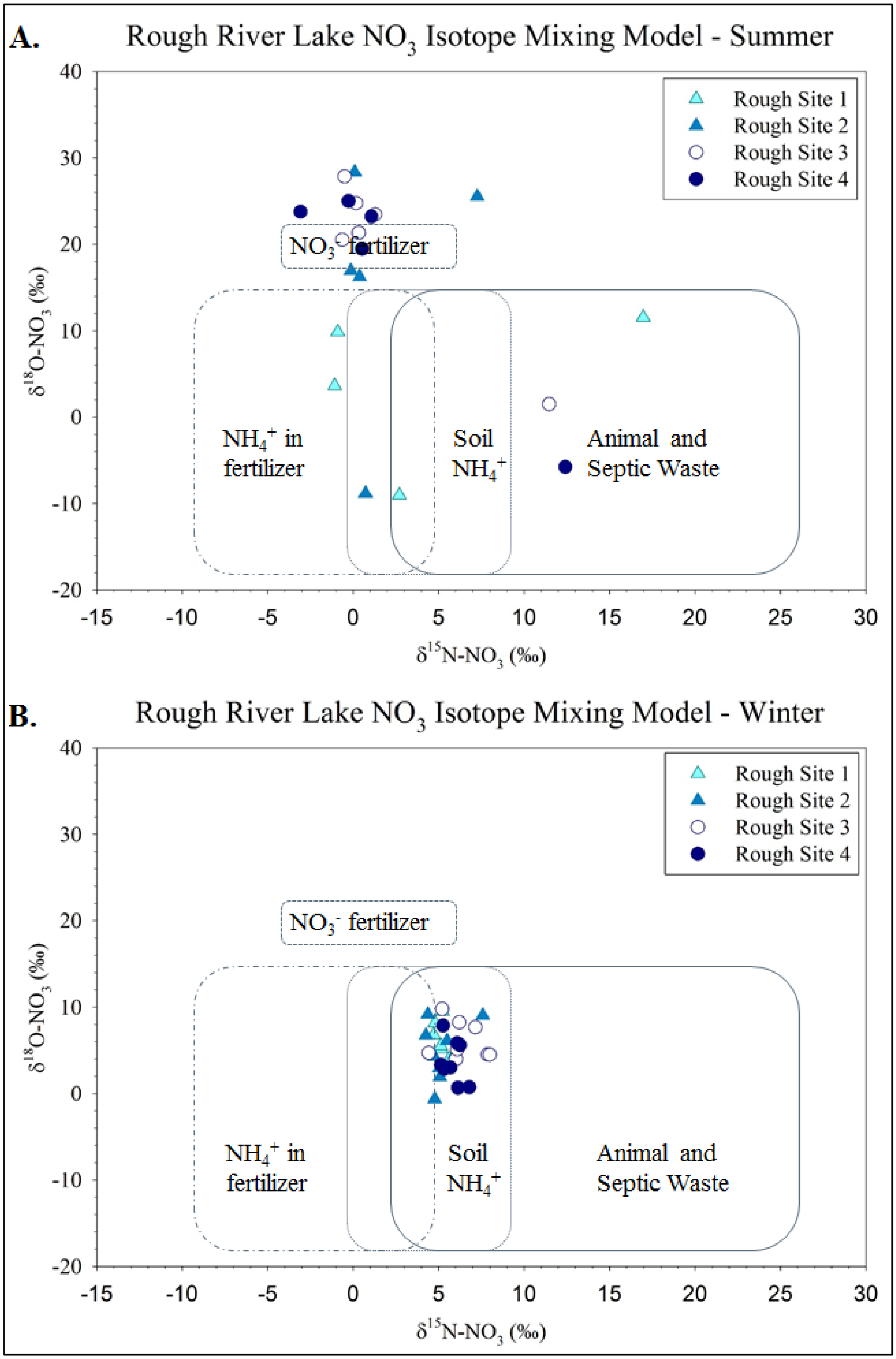



Figure 3.15. The mixing model for Rough River Lake. A) Summer, and B) winter, $\delta^{18}O\text{-}NO_3$ and $\delta^{15}N\text{-}NO_3$ values. These dual isotope mixing models were modified from Kendall et al. (2007).
Source: created by author, with modification from Kendall et al. (2007).

Site 2 (Laurel Branch Camp Grounds) had both inorganic $NH_4^+$ and synthetic $NO_3$ fertilizers being input during summer pool ($\delta^{15}N$ +0.37‰ to +7.26‰; $\delta^{18}O$ -8.8‰ to +28.36‰) and solely natural soil $NH_4/NO_x$ during winter pool ($\delta^{15}N$ +4.26‰ to +5.08‰; $\delta^{18}O$ -0.63‰ to +9.17‰) (Figure 3.15a-b). The land use around this site was, as the name suggests, a camping site, as well as a long-term recreational vehicle (RV) community, thus making the abundance of fertilizers surprising, unless the groundwater inputs are coming from elsewhere and have not been clearly identified. Site 2 had blue holes, as identified by the KGS karst map data, within the adjacent main channel of the lake and springs in the local area (KGS 2003; 2004). There was also an interesting record of an unidentified gas leak from under the sample site's sediments during the summer pool.

Site 3 (Forest Lake) and Site 4 (Peter Cave) had similar mixtures of nitrate contributors, which is interesting since they both represent main tributaries of the lake, but are not connected by surface connections. During summer pool, nitrate fertilizers were the main nitrate sources with animal/septic waste also providing a contribution. The summer $\delta^{15}N$ values ranged from -3.07‰ to +12.41‰ and $\delta^{18}O$ ranged from -5.76‰ to +27.84‰ between the two sites (Figure 3.15a). The winter values shift to natural soil $NH_4/NO_x$ and septic/animal waste, with the primary $\delta^{15}N$ values ranging from +4.42‰ to +7.99‰ and $\delta^{18}O$ ranging from +0.73‰ to +9.78‰ (Figure 3.15b). Site 3 has residences on either side of the lake, with sandstone bluffs beyond them, and farther from the lake there is a cluster of agricultural practices; this is also the case for Site 4. The only major difference being that Site 4 has a small campground and marina downstream of the sampling site (Figure 3.4). Again, the soil nitrate isotope numbers indicate that they are agricultural in origin (Kendall 1998).

For both Nolin River Lake and Rough River Lake, the nitrates are being introduced from a wide variety of non-point pollution sources. Interestingly, the landuses that are associated with these sources are rarely adjacent to the site, or even within the local area. Also, the shift from the randomness of the summer data to the tight clustering of the winter for both lakes is intriguing (Figure 3.14b and 3.15b). This shift could have occurred for many reasons but, since the shifts occur during the same timeframe, it suggests climatic, environmental, or lake/drainage basin dynamics are at play. The lakes are largely human controlled and with the drastic change in discharge and water level, a concentrating effect on the nitrates, as well as flushing effect from the local hydrogeology, could all explain this shift in the seasonal isotope data. The change in discharge could attribute for this clustering of the winter data, since the residency times decreased and water column mixing increased with the water level drop to winter pool. The shift is largely seasonal in nature, so environmental factors could also be an influence in the amounts of active bacteria in the soil, groundwater, and surface water. Higher amounts of bacteria acting on the dissolved nitrates, particularly those that were bound in the soil and flushed into the lakes through karst and hydrological processes, would produce similar $\delta^{15}N$ values (-3‰ to +15‰) as those recorded during this time period (Kendall 1998; Kendall et al. 2007). The mixing can be seen in the other datasets as well, with all of the values increasing and becoming more erratic with the onset of winter.  It is likely that a combination of these can be attributed to the combined winter clustering at both lakes.

While this clustering effect may be accounted for, the unexpected shift from fertilizers in the summer months to animal/septic waste and soil $NH_4/NO_x$ in the winter months is not. Given that the majority of the local land use of both lakes during the summer months is recreational in nature, a waste and soil $NH_4/NO_x$ signal detected in winter, and not summer, is surprising. This unexpected shift in the data could be attributed to similar reasons as the clustering. The anthropogenically controlled seasonal levels of the lakes likely play a role, but the degree of influence is unknown and a subject for further study. In fact, the dams' contribution to the effect of nutrient retention and mixing is entirely unknown and should also be studied in the future. Another surprising result was the lack of atmospheric nitrate contributions. With the increase in human activity around the lakes in the summer (i.e., burning fossil fuels), one would expect to see at least some contribution from these atmospheric sources as well. The variation of the local land use from the nitrate sources detected within this study further support that the nitrates are being introduced to the sites through a combined process of surface and subsurface drainage, with karst groundwater being highly influential, since it can provide input from further out in the basin and move quickly into the lakes through springs and blue holes.

This variance of detected sources from those available locally highlights the importance of using alternative sourcing and monitoring methods in karst regions to detect and understand potential inputs related to HABs. Targeting the local landuses will not address the nutrient pollution issue fully without an understanding of the extent and sources from which they originate. Another important lesson learned from these data is that the impacts land use has on a lake landscape can be myriad in nature, including those occurring outside the immediate vicinity of the lake. To affect change in these landscapes, broad ranging approaches are needed and any nutrient reduction plan that is developed should take into account all of the potential sources of nutrient input. These considerations are relevant, since the currently accepted practice to reduce HAB occurrence in freshwater environments is to limit nutrients from entering the system and, thereby, starve out any potential HAB before it occurs (Paerl et al. 2001; Paerl 2008).

**Monitoring/Managing HABs in Karst Regions:** Cyanobacteria have a set of specific conditions under which they are more likely to bloom. These conditions include parameters like temperature, sunlight availability, nutrients, and other water conditions. *Cylindrospermopsis raciborskii* needs water that is warm (15-35 ˚C, with optimal blooming at 25-30 ˚C), higher in pH (pH > 7), turbid, and has an abundance of nutrients (Saker and Griffiths 2000; Briand et al. 2004; Hamilton et al. 2005; Yamamoto and Nakahara 2005). To prevent a bloom from occurring, one must attempt to reduce or eliminate each of these conditions. At this moment, parameters like weather, climate, and the environmental conditions of the water are beyond human control, so the only truly effective approach is to reduce the overabundance of nutrient availability from anthropogenic sources. To this effect, a successful management and monitoring program against CyanoHAB formation in Nolin River Lake and Rough River Lake would be the best approach and need to be a multifaceted, proactive one that includes consideration for the karst groundwater influences.

The first step in this proactive, multifaceted approach should involve an active monitoring program, as indicated by this study. A better understanding of the karst geology should be established and, as such, continued monitoring using the same protocols developed in this study is advisable. Active monitoring at the lakes could improve the Kentucky Division of Water (KDOW) and USACE's response time in detecting and mitigating the potential damage CyanoHABs could inflict upon the water resources. As of June, 2015, the KDOW adopted a new management policy for HABs that relies upon the use of remote sensing to detect toxic CyanoHABs. This approach is only reactionary, because action is only taken after a HAB has been detected with the use of optical sensing technology or a HAB is visually detected and reported to KDOW. The problem with this approach is multi-fold. Not all cyanobacteria bloom in concentrations or ways that are visually detectable, even when they are producing potentially harmful toxins. Also, the toxins associated with the cyanobacteria, are not always local to CyanoHABs (USGS 2012). Once the toxins are released, either through excretion or cell lysing, the toxin is then free to mix in the water, without any visual cues. It then follows that the best way to mitigate any potential harm a CyanoHAB may have on people and animals is to actively try to prevent them (through nutrient reduction programs) and actively monitor for them, and if possible, predict the HAB's occurrence.

This active monitoring protocol should include all the basic geochemical parameters (SpC, pH, temperature, turbidity), biological markers (DO and *E. coli*), cations ($Ca^+$, $K^+$, $NA^+$), and anions ($NO_3$, $NO_2^-$, $PO_4$). These parameters should either be collected at this study's resolution, or even a higher-resolution sampling schedule, to capture storm pulses and rapid changes in the system to better characterize the local karst aquifers. This approach would allow for more proactive management, because the conditions for CyanoHAB could be identified when they occur and the costly monitoring for cell counts and toxins could commence in a more targeted manner. Other analyses that could be added to future studies include alkalinity and bicarbonate ($HCO^-$), since both of these parameters can be used to help better delineate the karst groundwater from the surface runoff. Building on the database established here could help the local resource managers gain a better sense of how karst groundwater influences and interacts with the lakes. Continuing the dual isotopic tracing efforts will help further the understanding of the seasonal dynamics of the nutrients in this karst riverine system. As this study showed, there are great seasonal fluctuations in this data and many non-point sources to target for any possible nutrient reduction programs. From this knowledge base, plans and policies could be developed.

Near the end of this study, the USGS set up several monitoring sites around the dam at each lake using YSI water quality sondes. The water quality parameter data gathered at these sites are based off the findings herein and likely will help to inform USACE personnel to potential HAB threats. These parameters are collected at a 15-minute interval and include temperature, turbidity, pH, DO, chlorophylls, phycocyanins, and SpC. Health Canada (2002), Canada's governing body responsible for its water security, developed a set of guidelines for HAB monitoring and response, which were later adopted by the WHO. These guidelines could likely prove to be the most cost effective way at monitoring and reacting to a CyanoHAB formation (Health Canada 2002; WHO 2011); they outline a multistep approach to monitoring and reacting to CyanoHABs, but do not have specificity related to karst regions. Similar to what is suggested here, the WHO study suggests to start with monitoring nutrient concentrations, then move to cell counts when the nutrient concentrations are greater than a set level (for $NO_3$ and $PO_4$), and finally toxin studies when the cells counts are high enough. This approach only calls for the active monitoring of the basic water quality parameters, but the results here add a few more parameters and sampling methods that better fit karst regions. An involvement of the local communities through the use of citizen science could also greatly improve the monitoring efforts by providing an avenue for education and increasing awareness about the issues.

Currently, the Friends of Nolin Lake, a non-profit organization, has a grant funded by the EPA 319b program, with the intent of using it to initiate a proper septic maintenance and lakeside management education program. This program is geared towards educating the local stakeholders in proper maintenance of their septic systems and repairing them in the event of leakages. The program also aims to help improve the lakeside land use and landscape through the implementation of littoral zone restoration projects. This program relies heavily upon social media and short informative internet-based videos to disseminate the information. Expanding this program to include incentives for the local citizenry to update and/or upgrade septic systems would likely not only greatly increase the health of the groundwater but also aid in limiting the non-point contamination occurring from this source. The USACE needs to be closely tied to these projects, because showing greater involvement in the local community and helping where assistance is needed in the form of incentive programs could help build relations between the local stakeholders and help foster a sense of community, which is needed in order to combat problems of this scale. Citizen science could also play a role in combating the HABs of these lakes. Having a social media presence and a geotagging-supported reporting process for any HABs within the lake would greatly help the monitoring of any HABs that occur with the lakes, as they are too large to monitor completely.

Another avenue of future research exists in a limnological survey of the karst-influenced lakes of Kentucky. The cyanobacteria that inhabit these lakes display unusual properties, such as the ability to bloom during winter months under ice. The water temperatures under the ice are within acceptable ranges for the temperate species of cyanobacteria that are native to Kentucky lakes, but are just below that of the sub-tropical invasive *Cylindrospermopsis raciborskii*; yet, it is this species that dominates winter cell counts. Research is needed to determine if the strains of the invasive CyanoHAB-forming cyanobacteria in these Kentucky lakes are specially adapted to the more temperate climate and, if so, what other changes might have occurred to these species.

As the land management programs gains success, the main contributors to nutrient pollution may shift and the management efforts need to follow that shift. Besides nitrate isotope tracing, examining the genetic origins of the *E. coli* could also indicate where the septic and animal waste components of the nutrient pollution are coming from, allowing land-use managers to better target their policy reforms.

Given the abundance of fertilizers entering the system during the summer months at both lakes, a more robust program aimed at educating not just the local producers around the lake, but all of the agricultural operations in the watersheds, should be initiated. The program should focus on educating farmers about proper application times and amounts of fertilizers in karst regions. Special care should be taken to inform those in the sinkhole plain about the sensitivity of their direct influence on the groundwater. Stressing the importance of buffer zones around sinkholes/fields, proper use of cover crops in the winter, and soil health should be the aims of this program. The USDA's Natural Resources Conservation Service (NRCS), working in concert with the Kentucky Department of Environmental Protection (KDEP) and the Kentucky Department of Agriculture (KDA), could augment these activities with already established programs

(Whitman et al. 2011). It is this combined effort of active monitoring, community water quality education and involvement, and septic/agricultural education outreach that is what will likely to have the widest reach and longest lasting impacts.

### 3.5 Conclusions

There exists a major karst groundwater influence on the occurrence of HABs in lakes in central and south-central Kentucky. This influence is confirmed through geochemical analysis of the surface water tributaries feeding Nolin and Rough River lakes, as well as in the concentrations and fluxes of nutrients and contaminants being transported through the system from surface activities. A combination of the geochemical and weather data revealed that karst groundwater is influencing conditions that favor cyanobacterial blooms (Figure 3.10 and 3.12). This is especially true for *Cylindrospermopsis raciborskii*, which is a cyanobacteria that bloomed repeatedly at both lakes according to the 2013 HAB survey data, including during the winter months at Nolin River Lake. The surface water temperature data at Nolin River Site 3 showed the dichotomy and seasonality of surface water temperature influences and karst groundwater effects. The $R^2$ of 0.85 ($p= <0.05$) for the summer average daily temperatures versus the surface water temperatures and $R^2$ of 0.55 ($p= <0.05$) for the winter temperatures (Figure 3.9) indicates that the karst groundwater has a stronger influence on temperature in the winter months as the volume of the surface water decreased with the lowering of the lakes to winter pool. This provides another line of evidence that karst groundwater's influence was detected in the waters of both lakes.

Along this line of thought, another potential influence on the nutrient concentrations and HAB occurrences is the human-controlled seasonality of the lakes.

The effects of the lake drawdown from summer pool to winter pool is clear in Figures 3.10 and 3.12 after Julian Date 334 (November 30, 2015). After the lakes were decreased to winter pool, nutrient data spiked, maxing out at 12.91 ppm $NO_3$ and 5.19 ppm $NO_3$ for Nolin River Lake and Rough River Lake, respectively. Other studies of reservoirs with nutrient loading indicate that low flushing rates and stratification can contribute to nutrient build-up in the sediments (Dillon 1975; Stanley and Doyle 2002). Further studies into this phenomena should be undertaken so that possible management actions can be informed and developed to help reduce the amount of nutrient build up in the lakes, thereby reducing the level of eutrophication they are currently experiencing.

A large amount of this nutrient pollution is entering the lakes through karst tributaries. Possible karst groundwater pulses were observable in the nutrient and geochemical data in response to storm events (Figure 3.10 and 3.12). Approximately 3,500,000 kg of nitrates went through Nolin River Lake during the eight months of this study (Table 3.3). Meanwhile the eight-month total nitrate load for Rough River Lakes was approximately 1,100,000 kg (Table 3.3). Both of these numbers are high and point to an issue of eutrophication for both lakes caused by karst groundwater inputs transporting contaminants from both near and far within the watersheds of both lakes.

The dual isotopic analysis of the heavy isotopes of $NO_3$ revealed that both lakes experience different non-point nutrient polluters and there even appears to be a seasonality to the main contributors of said pollution. Nolin River Lake experienced more fertilizers than animal/septic waste in the summer months, which then shifted to more natural soil nitrates and animal/septic waste contributors for the winter months (Figure 3.14). Rough River Lake also experienced a seasonal shift in the main contributors of its nitrate pollution with a mix of sources in the summer months that shift toward more soil based nitrates and animal/septic waste (Figure 3.15). Interestingly, most of these main contributors did not match up with the local land use of the immediately surrounding area at each site, further supporting evidence of the karst groundwater delivery of these nitrates and associated nutrients and contaminants.

Through the use of isotopic tracing, geochemical monitoring, and monitoring of bacterial and nutrient contaminants, it is possible to create a picture of the health of the lake ecosystem and, by proxy, understand the conditions under which CyanoHABs may form in karst influenced lakes. This allows resources managers not only to assess quickly and mitigate CyanoHABs as they form, but be proactive to suppress their occurrence in the first place. This multifaceted approach is what is needed to combat the issue of CyanoHABs in Kentucky's karst areas. Although more research needs to be done to shed light on the specific sources of nutrient pollution at each lake, additional planning and polices could even benefit more areas than just Kentucky, as HABs are found in all 50 states of the U.S., of which over 40% contain karst areas that may influence HAB occurrences from water conditions derived from karst hydrologic inputs.

# Chapter 4: Broader Impacts

HABs are dangerous to both terrestrial and aquatic life and threaten human health with more than just their toxins. HABs also negatively impact the economy around the lakes and bodies of water in which they occur, because the issuance of water use advisories may cause people to be less likely to visit those areas. The associated loss of tourism and economic activities, like fishing, can have a detrimental impact on the local communities and an overall negative effect on the quality of life for those living in the surrounding area. Throughout the course of this study, the data analyses have revealed that karst groundwater not only plays a role in the recharge, but also in the nutrient loading of these karst-influenced lakes. Evidence also exists to argue that the nutrient loading and geochemical effects these karst waters have on the surface waters aid in the formation of cyanobacterial-based harmful algal blooms. Armed with this information, a multi-agency task force should be assembled to develop plans of action against HAB formation for Rough River Lake and Nolin River Lake, as well as develop similar studies for other at-risk reservoirs in Kentucky and karst areas. It is the hope of this author that this study can be used in the development of a comprehensive plan for detecting and sourcing anthropogenic nutrient pollution and contaminants related to karst inputs, developing policies and plans to limit that pollution, lowering HAB occurrence and formation, and improving the health of the reservoir and its local residents.

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