Using Sediments to Identify Drivers of Cyanobacteria, Cyanotoxins, and Eutrophication in the Shallow, Subtropical Lakes of Florida, USA

by

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Abstract

Nutrient eutrophication and cyanobacteria dominance has increased in lake systems throughout the world over the last two decades, with growing concerns over decreases in ecosystem services and the toxins that some cyanobacteria produce. Monitoring of cyanotoxins and regulation of external nutrient inputs into water bodies have been enacted to mitigate increases in harmful algal blooms, but infrequency of sampling and the need to add additional measurements not normally afforded to monitoring programs, suggests the need for other analyses capable of longer timescales and other measurements. This thesis utilized the analysis of microcystin concentrations and paleolimnological measurements to accomplish two primary objectives: 1) determine the relationships of microcystin with nutrient, biological, and morphological variables to define the triggers that favor microcystin production, and 2) characterize changes in the past ~150 years in nutrient concentration, nutrient stoichiometry, and primary productivity through sediment samples of Florida lakes. Surface sediment samples (1-5 years) and deeper core samples (~150 years) were collected from a large sample size of lakes (n=47) throughout the state of Florida, USA. Four measured parameters (Fe, P, aphanizophyll, and secchi depth) demonstrate statistical relationships with total microcystin concentration and could serve as potential triggers of microcystin production. Most notably, stoichiometric ratios of Fe:P had the clearest direct relationship with microcystin concentrations, but Fe is rarely included in monitoring programs where eutrophication and/or cyanotoxin occurrence is of concern. This study demonstrates the potential to temporally reconstruct microcystin production from surface sediment samples and subsequently the drivers of microcystin production. In the temporal comparison of sedimentary and water nutrient concentrations, the differences in

nutrient trends suggest that even though external nutrient inputs have decreased, nutrient concentrations in the sediment could be internally loaded and sustaining phytoplankton populations through resuspension events. The analyzed subset of Florida lakes appears to have gone through much less limnological change in the past 40 years when compared to the 100 years prior, suggesting that substantial ecological shifts might have preceded monitoring data.

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List of Abbreviations

ANOVA	Analysis of variance
C:N	Carbon-nitrogen ratio
DOC	Dissolved organic carbon
EPA	Environmental Protection Agency
Fe	Iron
Fe:P	Iron-phosphorus ratio
GC-MS	Gas chromatography-mass spectrometry
HAB	Harmful algal bloom
HPLC	High performance liquid chromatography
ICP	Ion-coupled plasma
LC-MS	Liquid chromatography-mass spectrometry
MY	Microcystin
N:P	Nitrogen-phosphorus ratio
P	Phosphorus
PCA	Principal component analysis
S	Sulfur
SPE	Solid phase extraction
TC	Total carbon
TOC	Total organic carbon
TN	Total nitrogen

Chapter 1: Cyanobacteria and Cyanotoxins in Water and Sediment

Cyanobacteria and Cyanotoxins

Cyanobacteria are photosynthetic prokaryotes capable of out-competing other plankters in freshwater ecosystems to form dense communities known as harmful algal blooms (HABs). HABs can cause a variety of environmental problems such as oxygen depletion, fish kills, off-flavors, and cyanobacterial derived toxins called cyanotoxins (Pearl and Huisman 2009). During the last two decades, cyanobacteria dominance has increased throughout the world (Ewing et al. 2020, Taranu et al. 2015), causing greater concern for waterbodies where humans and HABs intersect (Bullerjahn et al. 2016, Carmichael and Boyer 2016). Cyanotoxins are secondary metabolites used for organismal purposes outside of primary metabolism (Holland and Kinnear 2013) and can negatively impact ecosystems and human health via bioaccumulation within organisms, oxidative stress of cells, and poisoning to various degrees of harm, including death (de la Cruz et al. 2013, Dittman and Weigand 2006, Kaplan et al. 2012). Concern regarding cyanotoxin production is increasing with many aquatic monitoring programs now including cyanotoxin measurements (EPA, LAKEWATCH). Cyanotoxins are found throughout all continents, climate zones (tropical, subtropical, temperate, and polar) and trophic states (mesotrophic, eutrophic, and hypereutrophic) (de La Cruz et al. 2013, Kinnear 2010, Rzymski and Poniedzialek 2014). Whereas the production of cyanotoxins typically occurs during times of increased primary production (Rzymski and Poniedzialek 2014), the specific ecological triggers causing cyanotoxin production are still unknown. However, the increasing occurrence of cyanotoxins indicates that the ecological triggers that induce cyanotoxin production are becoming more common (Paerl and Huisman 2009).

Of the multitude of cyanotoxins known, the most frequently measured in aquatic samples is microcystin (Kaplan et al. 2012). Microcystin (MY) is predominantly produced by freshwater strains of cyanobacteria, including some filamentous, nitrogen-fixing genera (Dittman and Weigand 2006, Kaplan et al. 2012). In higher biota, microcystin acts as a hepatotoxin, accumulating from the small intestine into the liver where it inhibits protein phosphatases 1 and 2A, damages liver cells, and promotes tumors (Kaplan et al. 2012). With more than 250 known variants of microcystin, most microcystin congeners are structural variations of seven amino acids coupled with changes in the structural side groups (Dietrich and Hoeger 2005, Dittman and Weigand 2006). The congener microcystin-LR, defined by leucine and arginine amino acids within the molecule, is the most frequently studied variant of microcystin due to its ubiquitous occurrence in eutrophic lakes and its relatively extreme toxicity (Dittman and Weigand 2006).

Cyanotoxin Ecology

Observational studies and experiments have investigated the environmental triggers of microcystin production, but results vary and are generally inconclusive. While environmental conditions (e.g. temperature, light, and pressure of predator grazing) and nutrient concentrations are known drivers of HABs, triggers of microcystin production specifically have not been as straightforward. Microcystin concentrations have correlated with increases in temperature suggesting that temperature regulates the release of microcystin molecules (Walls et al. 2018). However, Wiedner et al. (2003) documented that microcystin release resulted from a specific light threshold. With contradicting results, one study found that the production of microcystin occurred at pH above and below the range for optimum growth (van der Westhuizen et al. 1988), while a different study concluded a lack of pH effects on microcystin production (Jaiswal et al. 2008). Top-down impacts, such as predator grazing, have showed a limited ability to decrease

microcystin concentration in the water column (Oberhaus et al. 2007). Concerning nutrients, increases and decreases in nitrogen have been proposed as trigger mechanisms for microcystin production (Holland and Kinnear 2013). Phosphorus-limitation can cause microcystin production (Kurmeyer 2011), but studies contradict one another when evaluating the effect of iron. In fact, Utkilen and Gjolme (1995) recorded high microcystin production in high iron conditions, while Lukac and Aegerter (1993) found that high microcystin production correlated with lower iron concentrations. Sulfur-limited systems resulted in decreases in microcystin production over the entirety of the cellular life cycle suggesting a requirement for sulfur in microcystin production (Long 2010). Collectively, these studies demonstrate the complex and puzzling relationship between cyanotoxin production and direct environmental triggers used in many monitoring programs and experimental manipulations.

Stoichiometric ratios of essential nutrients for cyanobacterial growth (C:N, N:P) can be just as important as single nutrient concentrations in cyanobacteria assimilation efficiency and community structure (Wetzel 2001). Ecological resource theory suggests low N:P ratios favor cyanobacteria dominance because some cyanobacteria genera can fix nitrogen to outcompete other primary producers (Smith 1983). Some microcystin-producing species (e.g. *Nostocales*) are diazotrophic, and multiple studies demonstrate that low N:P favors higher microcystin production, though "low" N:P definitions vary (Harris et al. 2014, Harris et al. 2016, Orihel et al. 2012). Notably, not all microcystin-producing species are diazotrophic (e.g. *Microcystis*), thus other studies show the converse, with relatively "high" N:P favoring the production of the nitrogen-rich microcystin (Dolman et al. 2012, Mowe et al. 2014, Scott et al. 2013, Van de Waal et al. 2014, Wagner et al. 2019). Availability of inorganic C was shown to effect C:N stoichiometry and microcystin composition (form of microcystin congener) and production

within cyanobacterial cells (Van de Waal et al. 2009). Low C:N seems to correspond with high microcystin production (Van de Waal et al. 2009, Wagner et al. 2019). From physical, nutrient, and nutrient stoichiometry studies, it is apparent that high microcystin concentrations can occur both under optimal growth conditions and under various limitations. Overall, there is little scientific consensus on what environmental conditions promote microcystin production.

Cyanotoxin Measurement of Water and Sediments

Based on concerns over cyanotoxin presence and production, cyanotoxin measurements are frequently integrated into modern water monitoring programs, but little is known of cyanotoxin occurrence prior to these monitoring efforts. Because cyanotoxin measurements are expensive, monitoring frequencies are typically limited to intermittent samplings (monthly, annually) of a few locations (EPA, LAKEWATCH). Primary producers in aquatic systems (i.e. algae) respond quickly to environmental changes because of short reproductive periods. Environmental conditions in aquatic systems change rapidly from alterations in temperature, rainfall, wind, and other environmental variables. Thus, environmental variability confounds the representative nature of infrequent sampling and can complicate the interpretation of measured cyanotoxin occurrence. One avenue of cyanotoxin analysis and environmental assessment that incorporates longer timescales and more broad-scale and whole-ecosystem analysis is the application of paleolimnological techniques in the sediment record.

Paleolimnology utilizes sediment deposition as a long-term dataset representative of whole-lake environmental conditions. Paleolimnological analysis of sediment cores can produce data over longer temporal scales (i.e. annual, sub-decadal, decadal periods) thus preceding monitoring efforts for most lake systems. Sediment deposition integrates yearly samples related to long-term processes instead of daily environmental fluctuations. Cyanobacteria abundance has

been inferred using the paleolimnological tools of direct measurements, such as photosynthetic pigments (Leavitt and Hodgson 2001), and indirect measurements, like diatoms (Whitmore 1989) and stable isotopes (Brenner et al. 1999). However, very few studies have sought to utilize cyanotoxins as a paleolimnological proxy (Table 1.1) (Efting et al. 2011, Kaczorowska and Kornijow 2017, Pawlik-Skowronska et al. 2010, Waters 2016, Zastepa et al. 2017[1], Zastepa et al. 2017[2]). Matching cyanotoxins, specifically microcystin, with other sediment variables could lead to a better understanding of toxin ecology and production.

Though cyanotoxin measurements in sediments are not common, some paleolimnological studies have incorporated cyanotoxins (microcystin and cylindrospermopsin) as measured proxies (Table 1.1). When considering the status of establishing cyanotoxins as a paleolimnological tool, two conclusions arise. First, a variety of extraction techniques have used multiple solvents, time signatures, temperatures, and sediment amounts. Only a few extraction protocols applied solid phase extraction (SPE) for concentration of metabolites-of-interest and removal of interferents; solid phase extraction techniques (including SPE and solid phase adsorption technology) are recommended for optimal cyanotoxin recovery (Mashile and Nomngongo 2017). Second, multiple toxin analysis methods have been applied to extractions such as liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and Immuno-Assays (ELISA). Paleolimnological studies have been conducted on lakes in Poland, Nebraska, USA, and Canada to track eutrophication and microcystin production through time (Efting et al. 2011, Kaczorowska and Kornijow 2012, Pawlik-Skowronska et al. 2010, Zastepa et al. 2017[2]). The studies of microcystin deposition in the sediments have been limited to short (maximum of 50 cm) sediment-water interface cores that span the past ~150 years and studies of 1 to 3 lakes in an area. Most studies of sedimentary

microcystin have quantified total cyanotoxin concentration or specifically the MY-LR congener, however two of the studies quantified concentrations of 8 microcystin variants, including: MY-LR, -RR, -YR, -WR, -LA, -LF, -LY, and -LW (Zastepa et al. 2017[1], Zastepa et al. 2017[2]). MY-LR and MY-LA were the dominant forms of microcystin documented within the sediment samples (Zastepa et al. 2017[1]). MY-LA has been found to be less frequent but more persistent in aquatic environments (Beversdorf et al. 2017, Graham et al. 2010, Newcombe et al. 2003).

When compared to more established paleolimnological tools such as diatoms and photosynthetic pigment concentrations, these paleolimnological studies demonstrate the potential of sedimentary microcystin concentrations to reconstruct cyanotoxin production through time and demonstrate relationships to other established proxies such as nutrients, diatoms, and stable isotopes. Kaczorowska and Kornijow (2012) used microcystin measurements and chironomid head capsules in a sediment core from Poland to show that microcystin concentrations tracked with chironomid change. Only one study by Zastepa et al. (2017[2]) has attempted to directly document the relationships between sedimentary microcystin concentrations and other sedimentary variables. In Lake Baptiste, Canada, there was a strong positive correlation between microcystin concentrations, total Kjedahl N, and total P inferred from diatom analysis (Zastepa et al. 2017[2]). A paleolimnological study of a different cyanotoxin, cylindrospermopsin, in Lake Griffin, FL documented cyanotoxin concentrations in a 4700-year-old record supporting the ability of cyanotoxins to persist in long-term paleolimnological periods (Waters 2016). This study also utilized multivariate statistics (principal component analysis, cluster analysis) to show cyanotoxin relationships with many of the proposed nutrient triggers described above (Waters 2016).

Linking changes in microcystin concentrations to environmental conditions is confounded due to a lack of understanding of deposition, preservation, and measurement within the sediments. Microcystin has the potential as a paleolimnological tool, but little research has been conducted to calibrate and validate sediment core measurements with a standardized method as has been done with other paleolimnological proxies (Smol 2008). Because microcystin has numerous congeners, post-depositional transitions and transformations must be established to understand degradation pathways in the sediment. Henao et al. (2019) enumerates multiple scientific developments necessary for implementing cyanotoxins as a paleolimnological tool, including:

- Improve and standardize extraction techniques for cyanotoxins in sediments.
- Establish linkages between sediment cyanotoxin concentrations and water column cyanotoxin production.
- Document post-depositional transformations and degradation pathways of cyanotoxins in sediments.
- Compare concentrations of sedimentary cyanotoxins to other paleolimnological proxies.

Study Sites

Florida lakes form a unique limnological group due to their geologic, morphologic, and geographic characteristics. The geologic underpinning of the majority of Florida consists of limestone rock that, over time and in connection with water, creates sinkholes by two different mechanisms to ultimately form lakes (Brenner et al. 1990). In areas where the limestone is buried under clay, phosphatic mudstone, and peat, dissolution of the limestone causes a consistent collapse of the overburden. However, in areas in which the limestone is closer to the

surface, dissolution creates more abrupt, cavernous sinkholes (Wetzel 2001). The karst topography of Florida encourages the formation of many small lakes with only five natural lakes having a surface area greater than 100 km^2 (Wetzel 2001). Because the Florida landscape is dominated by thousands of lakes, each aquatic system is relatively isolated with small watersheds. Therefore, water inflow into Florida lakes is primarily from direct rainfall and groundwater and less from overland flow or contributing river and stream tributaries (Wetzel 2001). The underlying geology of central Florida also contains phosphatic minerals that can contribute to naturally high concentrations of soluble phosphorus in the water bodies. Florida lakes are subtropical and shallow (on the global scale). Shallow lakes are polymictic and discourage thermal stratification, though stratification can occur during the summer months from intense subtropical temperatures (Wetzel 2001). Warm temperatures, mixing that promotes internal nutrient loading, and long residence times result in extended growing seasons from primary producers and multiple biogeochemical pathways for nutrient dynamics.

Because of these natural characteristics and anthropogenic inputs of nutrients into aquatic bodies, Florida lakes are experiencing increased eutrophication at a noticeable rate (Ewing et al. 2020, Paerl and Huisman 2008, Riedinger-Whitmore et al. 2005, Taranu et al. 2015). Both federal and state-sanctioned legislation and regulation of external nutrient inputs into water bodies have been enacted in an attempt to mitigate these increases in algal blooms (Hudnell 2010). However, it is unclear whether efforts to minimize nutrient inputs into water systems have been effectual in decreasing aquatic nutrient concentrations. Florida lakes are a prime study location for temporal comparison and measurements of nutrients, eutrophication, and cyanotoxin concentrations.

Thesis Rationale and Objectives

Given the need to better understand microcystin drivers in lake systems, this thesis focused on applying paleolimnological tools including microcystin analysis to multiple Florida lakes. Surface sediment samples (1-5 years) and deeper core samples (~150 years) were collected from a large sample size of lakes (n=47) throughout the state of Florida, USA, which has known occurrences of microcystin (Figure 1.1) (Bigham et al. 2012). Microcystin, photosynthetic pigments, organic matter, and nutrients were measured on duplicate or triplicate samples from each lake forming a comprehensive dataset of whole-lake cyanotoxin production and nutrient dynamics over the last 1 to 5 years. Analysis of microcystin concentrations and other paleolimnological measurements were used to address two key objectives of the study:

1. determine the relationships of microcystin with nutrient, biological, and morphological variables to define the triggers that favor microcystin production in shallow, subtropical Florida lakes, and
2. characterize changes in the past ~150 years in nutrient concentration, nutrient stoichiometry, and primary productivity through sediment samples of Florida lakes.

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Tables

Table 1.1. Summary of cyanotoxin paleolimnological studies adapted from Henao et al. 2019.

Toxin	Location	Core Length[1]	Oldest Date[2]	Detection Method	Extraction	Reference
MC (8 congeners)	Canada, Lake Baptiste	50	1824 AD	LC-MS	75% MeOH, SPE	Zastepa et al. 2017[2]
MC-Total	Poland, Lake Głębokie	40	n.a.	GC-MS	75% MeOH	Pawlik-Skowronska et al. 2010
MC-Total	Poland, Lake Syczyńskie	50	n.a.	GC-MS	75% MeOH	Pawlik-Skowronska et al. 2010
MC (8 congeners)	Canada/USA, Lake of the Woods	7	2000 AD	LC-MS	75% MeOH, SPE	Zastepa et al. 2017[1]
MC-Total	Poland, Lake Syczyńskie	50	1800s?	GC-MS	75% MeOH	Kaczorowska and Kornijow 2017
MC-LR	USA (Nebraska), Two Mile Lake	18	1866 AD	LC-MS	EDTA, $Na_4P_2O_7$, MeOH, SPE	Efting et al. 2011
MC-LR	USA (Nebraska), Lake Dewey	40	1945 AD	LC-MS	EDTA, $Na_4P_2O_7$, MeOH, SPE	Efting et al. 2011
MC-LR	USA (Nebraska), Island Lake	20	1832 AD	LC-MS	EDTA, $Na_4P_2O_7$, MeOH, SPE	Efting et al. 2011
CYN	USA (Florida), Lake Griffin	300	4732 BP	ELISA	50% MeOH	Waters 2016

n.a.: not available; SPE: solid phase extraction; [1]core length in cm representing the deepest depth of cyanotoxin detection; [2]oldest date representing the oldest dateable section of the core with cyanotoxin detection

Figures

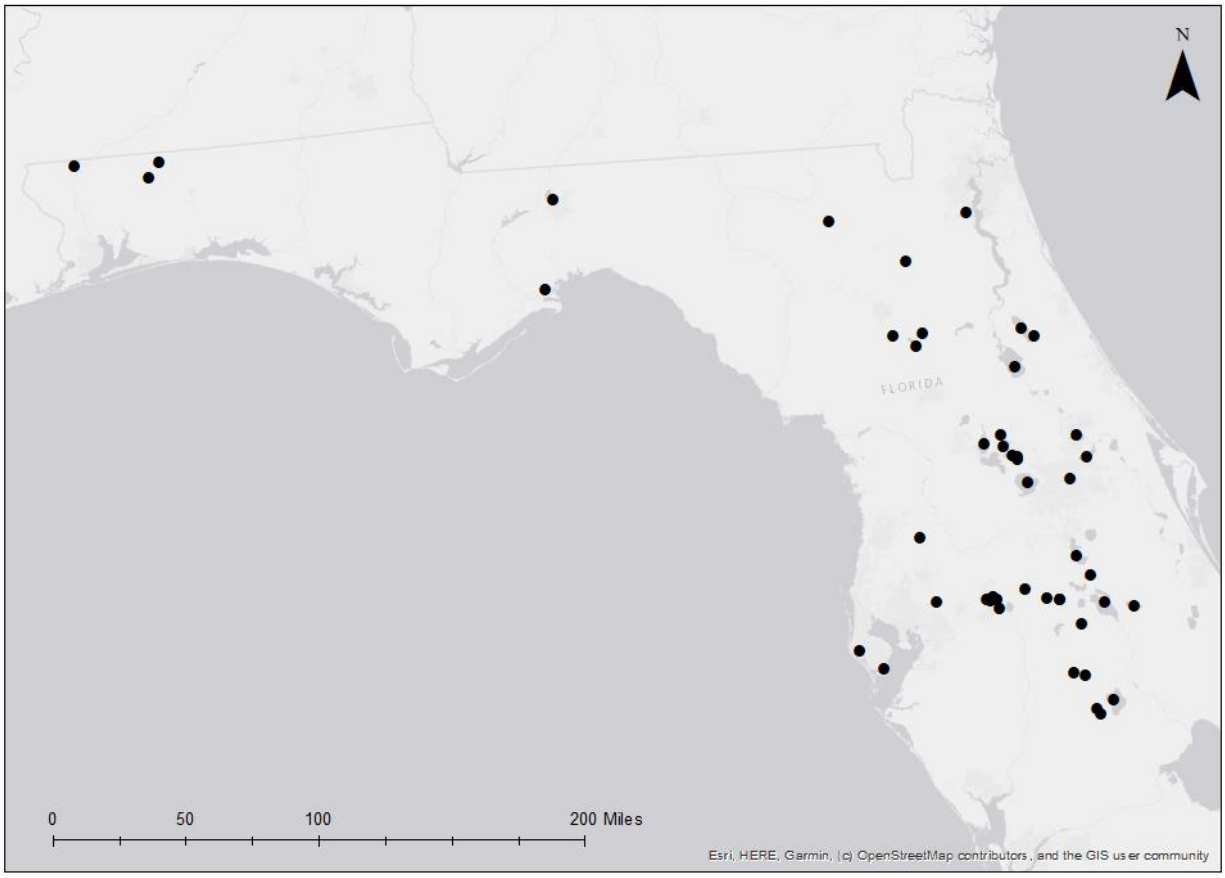

Figure 1.1. Map of Florida lakes (n=47) sampled for surface sediments and historic sediment core samples in July 2018.

Chapter 2: Using Sediment Analysis to Identify the Triggers of Microcystin Production

Abstract: Cyanobacteria dominance has increased in lake systems throughout the world over the last two decades with growing concerns over increases in cyanobacteria abundance and the toxins that some cyanobacteria can produce. Observational studies and experiments have attempted to determine the environmental triggers of cyanotoxin production, but results are generally inconclusive and often contradicting. This study collected water and surface sediment samples from 47 lakes in Florida, USA and applied sedimentary paleolimnological tools to 1) determine the geographic distribution of microcystin in the surface sediments of the study lakes and compare these values to water column measurements, and 2) identify the relationships of sedimentary microcystin values with nutrient, biological, and morphological variables considered triggers of microcystin production in shallow, subtropical Florida lakes. Four measured parameters (Fe, P, aphanizophyll, and secchi depth) demonstrate more marked possibility as triggers/indicators of microcystin production than other variables. Most notably, stoichiometric ratios of Fe:P had the greatest direct relationship with microcystin concentrations. Fe is rarely included in monitoring programs where eutrophication and/or cyanotoxin occurrence is of concern but can easily be extracted and measured within the sediments. The inclusion of sediment sampling in monitoring programs can provide a more temporally integrative picture of cyanobacterial abundance and cyanotoxin concentrations than that of intermittent water sampling. This study demonstrates the potential to temporally reconstruct microcystin production from surface sediment samples, and subsequently identify the drivers of microcystin production. Applications of these measurements could be to establish when cyanotoxin occurrence began in lake systems and what limnological characteristics seem to promote cyanotoxin production.

Introduction

Cyanobacteria dominance has increased in lake systems throughout the world over the last two decades (Ewing et al. 2020, Taranu et al. 2015) with growing concern over increases in cyanobacteria abundance and the toxins that some cyanobacteria can produce. These toxins, called cyanotoxins, are secondary metabolites that negatively impact human health (de la Cruz et al. 2013, Dittman and Wiegand 2006, Kaplan et al. 2012) and are a management target in waterbodies that are utilized for anthropogenic purposes (Bullerjahn et al. 2016, Carmichael and Boyer 2016). Whereas the production of cyanotoxins typically occurs during times of increased primary production (Rzymski and Poniedzialek 2014), the specific ecological triggers causing cyanotoxin production are still unknown (Henoa et al. 2019). Nutrient inputs from alterations in land use and warming global temperatures favor proliferation, dominance, persistence, and geographic distribution of cyanobacteria with increases in cyanotoxins projected to follow with synchronicity (Paerl and Huisman 2009).

Cyanotoxins negatively impact ecosystems and human health via bioaccumulation within organisms, oxidative stress of cells, and poisoning to various degrees of harm, including death (de la Cruz et al. 2013, Dittman and Weigand 2006, Kaplan et al. 2012). The four major cyanotoxins—microcystin, nodularin, saxitoxin, and cylindrospermopsin—are structurally and toxicologically diverse (Pearson et al. 2010). Microcystin (MY) is the most frequently reported cyanotoxin in freshwater environments, predominantly produced by freshwater strains of cyanobacteria (Dittman and Weigand 2006, Kaplan et al. 2012). With more than 250 known variants of microcystin, most microcystin congeners are structural variations of seven amino acids coupled with changes in the structural side groups (Dietrich and Hoeger 2005, Dittman and Weigand 2006). The congener microcystin-LR, defined by leucine and arginine amino acids

within the molecule, is the most frequently studied variant of microcystin due to its ubiquitous occurrence in eutrophic lakes and its relatively extreme toxicity (Dittman and Weigand 2006).

Observational studies and experiments have attempted to determine the environmental triggers of cyanotoxin production, but results are generally inconclusive and often contradicting. Microcystin concentrations have correlated with increases in temperature suggesting that temperature regulates the release of microcystin cells (Walls et al. 2018). However, Wiedner et al. (2003) documented that microcystin release resulted from a specific light threshold. With contradicting results, one study found that the production of microcystin occurred at pH above and below the range for optimum growth (van der Westhuizen et al. 1988), while a different study concluded a lack of pH effects on microcystin production (Jaiswal et al. 2008). Top-down impacts, such as predator grazing, have showed a limited ability to decrease microcystin concentration in the water column (Oberhaus et al. 2007). Nitrogen and P, commonly associated with eutrophic conditions, have been identified as key drivers of cyanotoxin production, but the relative importance of each is disputed (Dolman et al. 2012, Pick 2016). Increases and decreases in nitrogen have been proposed as trigger mechanisms for microcystin production (Holland and Kinnear 2013). Fluctuations in phosphorus concentrations can cause microcystin production (Kurmeyer 2011), but studies contradict one another when evaluating the effect of iron. In fact, Utkilen and Gjolme (1995) recorded high microcystin production in high iron conditions, while Lukač and Aegerter (1993) found that high microcystin production correlated with lower iron concentrations. Sulfur-limited systems resulted in decreases in microcystin production over the entirety of the cellular life cycle suggesting a requirement for sulfur in microcystin production (Long 2010). Some microcystin-producing species (e.g. *Nostocales*) are diazotrophic, and multiple studies demonstrate that low N:P favors higher microcystin production, though “low”

N:P definitions vary (Harris et al. 2014, Harris et al. 2016, Orihel et al. 2012). Not all microcystin-producing species are diazotrophic (e.g. *Microcystis*), thus other studies show the converse, with relatively "high" N:P favoring the production of the nitrogen-rich microcystin (Dolman et al. 2012, Mowe et al. 2014, Scott et al. 2013, Van de Waal et al. 2014, Wagner et al. 2019). Availability of inorganic C was shown to affect C:N stoichiometry and microcystin composition (form of microcystin congener) and production within cyanobacterial cells (Van de Waal et al. 2009). Overall, there is little scientific consensus on what environmental conditions promote microcystin production.

As the concern over cyanotoxins and specifically microcystin increases, cyanotoxin measurements are frequently integrated into water monitoring programs. However, little is known of cyanotoxin occurrence prior to these monitoring efforts (~1995 CE to present). Because cyanotoxin measurements are expensive, monitoring frequencies are typically limited to intermittent samplings (monthly, annually) of a few locations thus producing snapshots of toxin occurrence as practiced in monitoring programs such as Florida's LAKEWATCH (LAKEWATCH) and the EPA's National Lakes Assessment (EPA). While these datasets are highly valuable to the documentation of cyanotoxin occurrence, environmental variability confounds the representative nature of infrequent sampling and the ability to identify trends and triggers that drive cyanotoxin production. As a result, additional samplings are needed to provide integration over longer timescales that are more representative of annual or multi-annual cyanotoxin occurrence. One source of whole-lake sampling that represents these longer timescales are the surface sediments in lake systems and the application of paleolimnological tools typically utilized to reconstruct lake and watershed characteristics through time (Smol 2008). The sedimentary measurements of nutrients (C, N, P), photosynthetic pigments

(chlorophylls and carotenoids) and the cyanotoxins themselves provide whole-ecosystem analysis of cyanotoxin occurrence as well as information on the potential triggers of cyanotoxin production. Sediment deposition integrates yearly samples related to long-term processes instead of daily environmental fluctuations. Matching cyanotoxins, specifically microcystin, with other sediment variables could lead to a better understanding of its production and occurrence. When compared to more established paleolimnological tools such as organic matter content and photosynthetic pigment concentrations (Leavitt and Hodgson 2001, Waters et al. 2005), there is potential for sedimentary microcystin concentrations to quantify cyanotoxin abundance and demonstrate relationships to other established proxies, such as nutrients and ecological parameters. Thus, this study collected water and surface sediment samples from 47 lakes in Florida, USA and applied paleolimnological tools to accomplish two research objectives: 1) determine the geographic distribution of microcystin in the surface sediments of the study lakes and compare these values to water column measurements, and 2) to identify the relationships of sedimentary microcystin values with nutrient, biological, and morphological variables considered triggers of microcystin production in shallow, subtropical Florida lakes.

Methods

Study Sites

The 47 study lakes were located throughout the state of Florida, USA (Figure 2.1). To accomplish the research objectives of this study, study lakes were chosen based on previously known measurements of microcystin and microcystin-producing species from prior monitoring efforts (Bigham 2008, LAKEWATCH). Therefore, study lake selection included documented water column cyanotoxin concentrations, increased trophic status, and accessibility for sediment collection (Bigham 2008, Burns 2008, Dobberfuhl 2003, LAKEWATCH, Williams et al. 2001).

Lake physical characteristics, including latitude and longitude, surface area, mean depth, secchi depth, and trophic state show the variety of lake systems studied (Table 2.1). The density of lakes in the panhandle of Florida is substantially less than in the peninsula, and the distribution of sample sites is representative of this density difference (Figure 2.1). All locations are natural lakes (n=47).

Sample Collection

Surface sediment and water samples were collected during the summer of 2018. Sediment cores were collected using a piston-coring device that maintains an undisturbed sediment-water interface (Fisher et al. 1992). The top 4 cm of the sediment core were immediately sectioned and designated as the "surface sediment" sample. ^{210}Pb dates from previous sediment core studies of Florida lakes indicate that surface sediment samples are representative of the previous 2-10 years of sediment deposition (Brenner and Binford 1988). Collecting the top 4 cm of sediment as opposed to a smaller section was used based on previous paleolimnological investigations in Florida (Waters et al. 2015, Schelske et al. 2005, Brenner et al. 1999) and to allow for enough material for sediment analytical measurements. Surface sediments for many Florida lakes are flocculent, so the sample section of 4-cm also contains material that can be resuspended due to short-term environmental fluctuations (e.g. wind and fetch) in shallow basins (Scheffer 2007). Three sediment cores (i.e. three surface sediment samples) were collected from each lake as replicates. The three sampling locations within each lake were largely selected based on prior knowledge of the surface sediment distribution and available bathymetric maps. If prior work had not documented sediment distributions, sampling locations were then chosen based upon the location with the highest likelihood of organic matter accumulation in the lake. Surface sediment samples were stored on ice and in the dark until they were returned to the laboratory at Auburn

University. A wet aliquot of known volume was removed for bulk density and determination of organic matter content. The remaining samples were frozen, freeze-dried, and ground with a mortar and pestle for analysis. Freeze dried sediment samples were then analyzed for photosynthetic pigments, total organic carbon, total nitrogen, elemental concentrations, and microcystin concentrations.

From each study lake, water samples were collected using an integrated water sampler. Water samples were collected from the most centrally-located sediment coring site and were filtered within 24 hours of collection. The water filtrate was refrigerated for the analysis of microcystin and dissolved organic carbon (DOC). The water filters were stored in the freezer for analysis of microcystin and photosynthetic pigments.

Laboratory Analysis

Organic matter content of the sediment samples was calculated by loss on ignition (LOI) at 550°C in a muffle furnace for 3 hours and reported as a percent (Hakanson and Jansson 1983). Total phosphorus (P), total iron (Fe) and total sulfur (S) were measured on freeze dried sediment and in sample water filtrate by an ion coupled plasma (ICP) analyzer following the EPA Method 6010B. Total organic carbon (TOC) and total nitrogen (TN) on the freeze-dried sediment were measured using a combustion CHN Costech Elemental Combustion System. Carbon and N samples were acidified in HCl vapors for 24 hours to remove inorganic carbon prior to analyses. Dissolved organic carbon (DOC) in the filtered-water samples was measured on a Shimadzu TOC-L analyzer equipped with an ASI-L autosampler.

Photosynthetic pigments—including chlorophylls, chlorophyll degradation products, and carotenoids—were measured via high performance liquid chromatography (HPLC) coupled with a photodiode array detector and a fluorescence detector. Measurements of photosynthetic

pigments in both sediment and water samples followed the methods of Leavitt and Hodgson (2001) and Waters (2016). Photosynthetic pigments were extracted using a mixture of 80:15:5 acetone: methanol: water in a -20°C freezer for 16-20 h. After extraction, samples were centrifuged and filtered through 0.22 µm syringe filters to remove particulates. Pigment samples were injected into a Shimadzu HPLC system following the mobile phase and time sequence of Leavitt and Hodgson (2001). Chlorophylls and carotenoids were separated by a Phenomenex Luna C18 column. Chlorophylls and chlorophyll-degradation products were measured by a fluorescence detector, and carotenoids were measured using a photodiode array detector. Pigments were identified by retention times and comparison of spectra to known standards (DHI, Denmark). Pigment concentrations are in units of nmol g^{-1} organic matter for sediments and nmol L^{-1} for water samples and were calculated by comparison of spectra peaks to measurements of known standards. Photosynthetic pigments in the sediment, water filter, and water filtrate samples were measured as soon as possible following sample collection to avoid post-collection degradation.

The extraction procedure of microcystin from freeze dried sediment was modified from the Eurofins Abraxis method of microcystin extraction from soil (Eurofins). Sediment was extracted with 3:1 solution of methanol: water acidified with trifluoroacetic acid. Trifluoroacetic acid is also used to adjust the pH of mobile phases involved in analysis on the HPLC. Samples were twice extracted with the acidified methanol solution, sonicated in a water bath for 20 minutes, and centrifuged. The supernatants were collected and combined. Combined supernatants were concentrated to ~1.5 ml by evaporation under controlled heat and nitrogen flow on an Organomation N-EVAP 111 Nitrogen Evaporator. Extracts were diluted up to 6 ml with water, sonicated, and vortexed to dissolve all contents. Extractants were then passed

through Strata-X 33 µm polymeric reversed phase columns to remove interferents. Strata-X columns were conditioned with 6 mL of methanol followed by 6 mL of water. The sample was passed through the column, and then the column was rinsed with 2 mL of 5% methanol. All volumes of the rinse, sample solution, and methanol rinse were discarded. The sample was eluted through the column with 9 mL of 90% acetonitrile. Eluted sample solutions were again evaporated under heat and nitrogen flow and reconstituted with water to a consistent volume for analysis.

The sediment-microcystin extraction solution was appropriate for analysis in both Eurofins Abraxis ELISA kits and HPLC. HPLC measurements were used to verify the occurrence of microcystin based on known spectra, but values are not reported here. Some samples required dilution to fit the calibration curve of the provided ELISA kit standards. Water filtrate samples, water filter samples, and sediment samples were all measured for total microcystin concentrations given the vast array of microcystin congregates and to account for any post-depositional transformations. Total microcystin concentrations were measured using Abraxis ELISA kits for total microcystin. The ELISA kits cannot discern between microcystin congers and thus measure microcystin concentrations in microcystin-LR equivalents. Concentrations of microcystin were measured and described in this study as ng cyanotoxin g^{-1} organic matter of sediment.

Results

Most of the study lakes (n=47) were located on the peninsula of Florida (n=42), and the remaining five sample lakes are located within the panhandle of Florida (Table 2.1; Figure 2.1). Surface area of the lakes ranged from 0.11 km^2 to 198.07 km^2. Like the majority of Florida lakes, most of the study sites are shallow (average depth < 4.0 m). Only two lakes (Eustis, Placid)

exceed 4.0 m in mean depth. Data for mean depth was not available for six lakes. Secchi depth, a measure of water transparency, ranged from 0.40 m to 2.74 m with 32 study lakes having a secchi depth <1.0 m. Forty four of 47 sample lakes were classified as eutrophic or hypereutrophic based upon Florida LAKEWATCH classification, however the skew towards higher trophic status is expected as such lakes were targeted in this study (LAKEWATCH). Two study lakes did not have a published trophic classification (Table 2.1).

Microcystin was detected in all (n=47) sediment samples ranging from 0.32 to 674.12 ng g^{-1} org. The mean total microcystin concentration in the sediment samples was 63.28 +/- 114.62 ng g^{-1} org. Six samples (Bonny, Carlton, Glenada, Hollingsworth, Jesup, Marian) were above ELISA kit detection limit, and therefore concentrations were determined using an extended curve-of-best-fit as recommended by Abraxis. However, these values can be differentially inflated based upon proximity to the highest calibration point where there is a greater relative increase in concentration per unit of absorbance. Concentrations are within the range of established sedimentary values (Kaczorowska and Kornijow 2012, Pawlik-Skowronska 2010, Efting at al. 2011, Zastepa et al. 2017[1], Zastepa et al. 2017[2]). Spatially, higher concentrations of microcystin are found in the Florida peninsula than in the panhandle, but within the peninsula itself, there was no discernible spatial pattern (Figure 2.1).

Though all sediment samples had measurable microcystin, only 35 of 47 water samples had measurable microcystin using the ELISA kits. Of the water samples in which microcystin was present, the mean concentration was 0.1171 +/- 0.1144 ng L^{-1}. There is a very weak positive relationship between sediment and water microcystin concentrations (R^2=0.1344) (Figure 3.1).

Concentrations of sedimentary photosynthetic pigments diagnostic for cyanobacteria, canthaxanthin and/or aphanizophyll, were present in all 47 surface sediment samples. However,

study lakes do not necessarily have high concentrations of both cyanobacterial photosynthetic pigments concurrently. Chlorophyll-a concentrations, an estimate of total primary productivity, ranged from 0.75 to 148.14 nmol g^{-1} org with a mean concentration of 38.14 +/- 26.34 nmol g^{-1} org. Study samples had mean total organic carbon (TOC) content of 25.06 +/- 10.13%, mean total nitrogen (TN) content of 2.35 +/- 1.13%, and a mean total phosphorus (P) concentration of 1.88 +/- 1.15 mg g^{-1}. Concentration of additional nutrients measured in this study (Fe and S) have similar mean concentrations between 9.70% and 10.00%. Within all sediment samples, stoichiometric molar-ratios of TOC:TN had an average of 13.26 +/- 3.73, TN:P an average of 31.94 +/- 17.04, and Fe:P an average of 38.00 +/- 30.96. Correlational analyses were performed between all the measured sediment parameters believed to relate to microcystin production and microcystin concentration. Within the correlational analyses, TN ($p=0.015$) and Fe:P ($p=0.040$) were the only parameters significantly correlated with microcystin concentration (Table 2.2).

A principal component analysis (PCA) including environmental and morphological characteristics (mean depth, secchi depth, surface area), biological parameters (aphanizophyll, canthaxanthin, and chlorophyll-a), nutrient concentrations (TOC, TN, P, Fe, S), and microcystin concentration was performed to emphasize variation and highlight patterns within the dataset (Figure 2.4). Principal components (PC) 1 and 2 explained 25.8% and 16.9% of the variance, respectively. The eigenvector of microcystin concentrations were positively ordinated with eigenvectors of P and aphanizophyll, while negatively ordinated with eigenvectors of Fe and secchi depth.

An outlier analysis of microcystin concentrations was performed on the data set and yielded six possible outliers due to high microcystin concentrations: Bonny (214.53 ng g^{-1} org), Carlton (244.18 ng g^{-1} org), Glenada (347.03 ng g^{-1} org), Hollingsworth (180.74 ng g^{-1} org),

Jesup (123.86 ng g^{-1} org), and Marian (674.12 ng g^{-1} org). Thus, a subset of these six lakes was created and termed "high"; the remaining lakes were termed "low". "High" and "low" lakes were reanalyzed for significant correlations with the environmental parameters and compared to one another (Table 2.3). Secchi depth (p=0.0387), TOC:TN (p=0.0205), and Fe:P (p=0.0029) were significantly different between "high" and "low" lakes.

Discussion

The surface sediment data from the 47 study lakes in Florida, U.S.A. show that measurable microcystin concentrations are deposited in sediment environments (>0.35 ng g^{-1} org) and are detectable even when water column toxins are not measurable. Primary producers in aquatic systems respond quickly to environmental changes such as alterations in temperature, rainfall, and wind, causing the characteristics of primary producer behavior (i.e. cyanotoxin production) to be transitory. In the case of microcystin measurements, environmental variability and infrequent sampling confound the accuracy of capturing a representative picture of the aquatic system from a water sample. Only 35 of the 47 lakes had measurable microcystin in the water column despite the study focusing on documented cyanotoxin-producing systems, but all 47 sediment samples had detectable microcystin concentrations. Basing microcystin occurrence in an aquatic system solely on the water column concentrations thus risks assigning false negatives. Sediments not only offer a multi-year mean of environmental conditions, but variables of interest infrequently measured in the water column can be quantified in the sediment. Nutrients such as Fe and S that have possible effects on microcystin production, with Fe:P identified in this study, have not historically been integrated into monitoring programs (EPA, LAKEWATCH). However, comprehensive quantification of a suite of nutrients, including Fe and S, are routinely measured in sediments (Slomp et al. 1997, Waters et al. 2010). As a result, the addition of

sediment analyses to water-column sampling programs could provide on increased understanding of cyanobacteria dynamics and cyanotoxin production than traditional water samples.

The comparison of sediment microcystin concentrations to environmental variables allows the determination of sedimentary variables related to microcystin production. Whereas the identification of drivers of microcystin production has proven to be difficult (Holland and Kinnear 2013, Jaiswal et al. 2008, Lukac and Aegerter 1993, Utkilen and Gjolme 1995, van der Westhuizen et al. 1986), sedimentary analysis allows the consideration of nutrient, biological, and morphological conditions to be considered in concert. For example, the statistical identification of Fe and Fe:P to be related to microcystin production was shown in my data, but Fe is rarely included in water column monitoring efforts. As a result, surface sediment sampling could provide additional and valuable data in monitoring programs that further the understanding of complex microcystin production.

Nutrients

Phosphorus is frequently identified as a cause of cyanobacterial proliferation since freshwater systems are often characterized as P-limited (Dignum et al. 2005, Schindler 1974). Many water quality monitoring programs include P as a regularly measured and regulated parameter, especially in environments with documented microcystin production (EPA, LAKEWATCH). My 47-lake Florida data set had an average P-concentration of 2.33 +/- 1.15 mg g^{-1}, which is high compared to other hypereutrophic systems such as Lake Okeechobee, Florida (Engstrom et al. 2006) and Lake Taihu, China (Jinglu et al. 2007). In addition to human-derived P inputs, Florida has natural occurring P from the local geology which could also be adding to the high values in the sediment (Brenner et al.1990). Though not significantly correlated with microcystin concentrations, P is positively ordinated in the PCA with sediment

microcystin. This suggests possible interactions between P dynamics and cyanotoxin production (Table 2.2, Figure 2.4). According to a recent meta-analysis of more than 2,500 published global microcystin studies, P contributes to microcystin occurrence more than other environmental variables (Buley 2020).

While P inputs are of concern to most monitoring programs, the availability of P for metabolic consumption is of greater concern in most lake systems (Scheffer 2007, Wetzel 2001). Environmental P cycling includes no atmospheric component, thus the P introduced and present in the waterbodies remains until it is bioassimilated by algae, bacteria, and other primary producers, transported aqueously out of system, or bound and deposited into the sediments (Torres et al. 2014, Wetzel 2001). In the shallow lakes of Florida, large pools of sedimentary-P can be reintroduced into the water column by fetch-induced resuspension (Cyr et al. 2009). This results in a near-constant resupply of P into the water column that can promote cyanobacterial abundance and processes including cyanotoxin production (Moore et al. 1998). The constant internal supply of P could be confounding the relationship between P and microcystin values in my study lakes. Other lake systems lacking internal loading of P should still consider P as a potential driver and the lack of a P relationship could be an artifact of shallow lake systems (Scheffer 2007). Higher concentrations of P can promote cyanobacteria dominance because of their ability to outcompete other primary producers and utilize available P (Wetzel 2001). Increased cyanobacterial abundance could proportionally increase the number of species that are microcystin producing.

Associated with terrestrial inputs, Fe has been hypothesized to biologically affect microcystin concentrations via intracellular chelation, but studies have published differing conclusions (Utkilen and Gjolme 1995). Utkilen and Gjolme (1995), Amé and Wunderlin (2005), and Sinang

et al. (2015) demonstrated high microcystin production in high Fe concentrations, while Lukač and Aegerter (1993) found increased microcystin production in low Fe concentrations. These experiments and studies were not measured in the sediment but instead in experimental growing media and water bodies. Sedimentary Fe concentrations in my 47 lakes were not significantly correlated with microcystin concentrations but were negatively ordinated with microcystin in the PCA, supporting a possible mechanism of interaction between high microcystin and low Fe.

There were no significant relationships between Fe and microcystin or P and microcystin, but the molar ratio of Fe:P was significantly correlated with microcystin concentrations and both Fe and P ordinated with microcystin in the PCA, negatively and positively, respectively. Low Fe:P corresponded to higher concentrations of microcystin in the sediment suggesting potential biogeochemical processes between the two elements as a trigger for microcystin production. Additionally, "high" lakes had significantly lower Fe:P ratios than the subset of "low" lakes (Table 2.3). Orihel et al. (2015, 2016) documented mechanisms by which low Fe concentrations in the sediment can contribute to increased soluble, available P during mixing events that can encourage cyanobacteria abundance, including microcystin-producing species. P is often bound to Fe, Al, Mg, and Ca when the sedimentary redox potential is high (i.e. in oxic conditions), with Fe playing an especially important role in the availability of P (Saavedra and Delgado 2005). Iron's affinity for binding P in sediments by the adsorption of P to the surfaces of ferric oxyhydroxides and formation of ferrous-phosphate minerals is well documented (Hupfer and Lewandowski 2008, Katsev et al. 2006). Iron-rich sediments can thus inhibit the recycling of available P back into the water column and reduce the internal load from sediments during resuspension events (Orihel et al. 2016). When sediments in polymictic lakes have low Fe concentrations, the ability to bind P is reduced creating a "nutrient pump" that delivers

bioavailable nutrients, including P, into the euphotic zone to be bioassimilated by primary producers (Orihel et al. 2015). Not only can increased available P concentrations directly promote cyanobacteria abundance, the subsequent lowering of N:P ratios from the P-flux can proffer benefits to N-fixing cyanobacteria, many of whom can produce microcystin (Orihel et al. 2015). Because of their complex cycling, the interactions between Fe and P, including the stoichiometric molar ratio of Fe:P in the sediments, could account for a more synergistic effect on microcystin production than direct effects of Fe and P individually. The possibility of Fe and Fe:P effects on microcystin production merits further study from water-column experiments because of the rarity of Fe measurement inclusion in cyanotoxin monitoring.

Sulfur is a molecular requirement for microcystin biosynthesis, and one study has shown that S-limitation decreased intracellular microcystin concentration (Long 2010). Because of their proximity to the ocean, Florida lakes contain notable amounts of S. However, S was not significantly correlated nor ordinated with microcystin concentrations in my surface sediments. The presence of S from natural conditions and the addition from the bacterial reduction of sulfate in anoxic pore waters can interfere with Fe's ability to bind P. If S is present, Fe preferentially binds to S (instead of P) (Caraco et al. 1989, Chanton et al. 1987). But because neither elemental-S nor any stoichiometric ratio involving sulfur was statistically significant, the role of S in promoting microcystin production appears minimal in the shallow lakes of Florida.

The effects of nitrogen on cyanobacteria and cyanotoxins is investigated with a frequency similar to P. Nitrogen can not only have effects on primary productivity, but TN is also needed for the formation of the microcystin molecule itself (Dolman et al. 2012, Giani et al. 2005, Graham et al. 2004, Wilhelm et al. 2011). Sediment TN in our study was significantly correlated ($p=0.0151$) with microcystin concentrations but did not ordinate with microcystin values in the

PCA. These contrary results add to the complexity of the relationship between N and microcystin dynamics. Though freshwater systems are often believed to be P-limited, lakes that are P-enriched, such as some Florida lakes, can instead be N-limited (Dolman et al. 2012). Previous studies have documented contrary trends in microcystin production under aqueous TN-rich and TN-poor conditions. The effect of TN concentrations on microcystin production was often dependent upon the cyanobacterial species' ability to fix nitrogen (Gobler et al. 2016, Holland and Kinnear 2013). However, not all cyanotoxin-producing species are diazotrophic (Holland and Kinnear 2013). Also, nitrogen burial in the sediment does not typically result in long term storage, but TN can be made bio-unavailable by bacterial processes in the nitrogen biogeochemical cycle (Guildford and Hecky 2000). Because of its atmospheric component, the representation of measured sediment TN can be skewed depending on other external factors, including rates of microbial transformation that are not typically measured in sediment studies.

Stoichiometric ratios of essential nutrients for cyanobacterial growth can be just as important as single nutrient concentrations in cyanobacteria assimilation efficiency and community structure. There was no significant correlation or ordination between sediment TN:P and microcystin concentrations in my study. While a lack of relationship occurred in my sediment analysis, water column TN:P influences competitive interactions among phytoplankton species (Dolman et al. 2012). Ecological resource theory suggests low TN:P ratios favor cyanobacterial dominance because some cyanobacterial genera can fix nitrogen and outcompete other primary producers (Smith 1983). Some microcystin-producing species (e.g. *Nostocales*) are diazotrophic, and multiple studies demonstrate that low N:P favors higher microcystin production, though "low" N:P definitions vary (Harris et al. 2014, Harris et al. 2016, Orihel et al. 2012). Not all microcystin-producing species are diazotrophic (e.g. *Microcystis*), thus other

studies show the converse with relatively "high" N:P favoring the production of the nitrogen-rich microcystin (Dolman et al. 2012, Mowe et al. 2016, Scott et al. 2013, Van de Waal et al. 2014, Wagner et al. 2019). Though there are contradicting conclusions from aqueous TN:P studies, the prevalence of inquiry implies that further study is needed to clarify results. This suggests that TN:P might be better investigated by water column measurements and that sediment TN:P dynamics are still in need of further investigation.

Total organic carbon (TOC) can be introduced into the aquatic environment from autochthonous sources, internal loading and transformation of organic matter within the water body, and allochthonous sources, organic material originating from the terrestrial watershed. TOC in the sediment record is a reconstruction of past biota and system productivity. Mean TOC was 29.50 +/- 10.13%, indicating high residual organic matter in these very productive aquatic systems. Stoichiometric ratios of total organic carbon : total nitrogen (TOC:TN) can indicate the source of organic matter—phytoplankton (autochthonous), macrophytes (autochthonous), or land plants (allochthonous)—due to differences in concentrations of cellulose and lignin (Meyers et al. 1993). The TOC:TN measurements in my surface sediment samples, ranging from 10.45 to 13.2, represent primarily autochthonous organic matter origins and support field observations that Florida lakes are dominated by macrophytic and algal carbon-inputs (Brenner et al. 1999[2], Waters et al. 2019). Previous studies have documented increased microcystin production at low TOC:TN (Van de Waal et al. 2009, Wagner et al. 2019). However, our TOC:TN measurements were not significantly correlated with sediment microcystin concentrations, and neither TOC nor TN ordinated with microcystin. The lower TOC:TN values suggest that TOC in the sediments of our study lakes are more labile forms of carbon capable of promoting microbial processes as mentioned above.

Biological Characteristics

Sedimentary photosynthetic pigments have been used to infer lake eutrophication histories and changes in primary producer community structure (Waters 2005, Leavitt and Hodgson 2001, McGowan et al. 2005). In this study, I primarily focused on the analysis of three photosynthetic pigments: aphanizophyll, canthaxanthin, and chlorophyll-a. None of the measured pigments were significantly correlated with microcystin. Aphanizophyll, specifically associated with nitrogen-fixing species of cyanobacteria, positively ordinated with microcystin concentrations, while canthaxanthin, the more general indicator of cyanobacteria, and chlorophyll-a did not (Leavitt and Findlay 1994). A positive ordination with aphanizophyll does not assert that cyanobacteria capable of N-fixation are necessarily responsible for microcystin production, but simply that these species are present and could serve as an indicator of possible microcystin presence. The lack of association between microcystin and canthaxanthin and microcystin and chlorophyll-a reasserts that there is a discrepancy between equating increased primary producer and/or cyanobacteria abundance to increased cyanotoxin production (Sinang et al 2015). Not all species are capable of microcystin production, and even if cyanotoxin-producing species are present, not all individuals have the genes necessary for production or are utilizing the cyanotoxin genes (Rinta-Kanto et al. 2009).

Environmental and Morphological Characteristics

Secchi depth, a measure of transparency, can be affected by a mixture of suspended solids, dissolved organic matter, and phytoplankton in the water column (Swift et al. 2006). Mean secchi depth measured in my study of Florida lakes was 0.9 +/- 0.5 m, an average of 41.3% of the lake depth. The subset of “high” lakes had a significantly higher mean secchi depth (i.e. lower transparency) than the “low” lakes. Though not significantly correlated with

microcystin, secchi depth was negatively ordinated with microcystin concentrations in the PCA suggesting a weak relationship between decreased water transparency and increased microcystin concentrations in the sediment. Several mechanisms, working separately or in conjunction, could be responsible for this trend. Resuspension of nutrient-rich surface sediments into the water column due to shallow depth and fetch intensity can increase turbidity. Additionally, DOC can cause lakes to have a brown, tea-like appearance, inhibiting light penetration (Williamson et al. 2016). Williamson et al. (2016) showed that many lakes in the USA are moving toward a "murky" classification which associates increased phytoplankton with DOC in the water column. DOC can be produced as a biproduct of cyanobacteria cells or can be an indication of terrestrial nutrient inputs (Tessarolli et al. 2018). Regardless of its source, DOC can increase cell abundance in the water column because it can be assimilated by cyanobacteria and used for growth (Paerl 1978). Furthermore, both nutrient-rich resuspension and increased DOC can additionally favor increased algal and cyanobacteria abundance, and higher cell densities can decrease secchi depth measurements (Mihaljević and Stević 2011). Some toxin-producing species can specifically outcompete other phytoplankton in turbid environments by more efficiently assimilating available nutrients or positioning themselves higher in the water column by maintaining buoyancy and intercepting light (Paerl and Huisman 2008). Whereas my data do not support a direct, causal relationship between low water transparency and microcystin production, management programs might benefit by more closely monitoring particularly turbid systems for increasing microcystin concentrations.

Surface area and mean depth neither significantly correlated nor ordinated with microcystin concentrations. Though it is well established that surface area and mean depth contribute to frequency of polymixis and the resuspension of nutrient-rich sediments that become

available to algae, these two morphological conditions do not seem to directly relate to microcystin production in my study lakes.

Other Possible Drivers of Microcystin Production

While surface sediment analyses add to the variables investigated in microcystin systems, some proposed triggers of cyanotoxin production are not measured in my study. Increases in global temperatures favor the proliferation, dominance, persistence, and geographic distribution of cyanobacterial communities (Pearl and Huisman 2008). The concern of increased temperatures on cyanotoxin production stems from the possible enlargement of tolerable habitats as lakes warm (Pearl and Huisman 2009). Additionally, increases in temperature are associated with increases in microcystin because temperature can regulate the release of microcystin from toxin-producing cells (Walls et al. 2018). I attempted to elucidate any geographical trends, a proxy for temperature differences, but no discernible patterns were present in a spatial analysis of our measured microcystin concentrations (Figure 2.2). Even though our samples range latitudinally, the temperature gradient in subtropical Florida may not be steep enough to demonstrate temperature effects on microcystin.

Identifying Key Drivers of Microcystin

The key drivers that promote the production of microcystin by harmful cyanobacteria remain complex and confounding. Scientific experimentation and decades-long monitoring have not produced definitive explanations for microcystin production. Because this study utilizes surface sediment instead of water samples as a measurement medium, environmental variables that are either not frequently or not able to be measured in the water column can be compared concurrently. Based on this survey of 47 lakes in Florida, there do exist nutrient, biological, and morphological parameters that may independently or simultaneously promote microcystin

production. Four measured parameters (Fe, P, aphanizophyll, and secchi depth) demonstrate more marked possibility as triggers/indicators of microcystin production than other variables. As a proxy for N-fixing cyanobacteria, the association between aphanizophyll and microcystin concentrations suggest that lakes with larger proportions of diazotrophic species might be more likely to produce high concentrations of microcystin. Low secchi depth measurements may also be associated with increased microcystin as high levels of suspended solids, dissolved organic matter, and suspended algal bodies can differentially promote cyanotoxin-producing species. Most notably, stoichiometric ratios of Fe:P had the clearest direct relationship with microcystin concentrations, but Fe is rarely included in monitoring programs where eutrophication and/or cyanotoxin occurrence is of concern. There is a lack of scientific exploration on the ability of Fe to affect cyanobacteria abundance and microcystin production and a need to include Fe and P as regularly measured variables.

Conclusions

Management Implications

Lake monitoring programs typically focus on direct water quality measurements for ease and cost of collection. However, a case can be made that the benefits of integrative sediment records outweigh the labor and financial costs of sediment collection. I have established in my study that measurements of both Fe and P concentrations (Fe:P) have potential to be utilized as indicators of increased microcystin production. Fe is typically not measured in monitored water samples and can easily be extracted and measured within the sediments (EPA, LAKEWATCH). Already integrated into monitoring programs because of its ease of measurement in the water column, low secchi depth could be both an indicator and a promoter of microcystin concentrations. Both national (EPA) and state/local (LAKEWATCH) programs already utilize

secchi measurements with regularity. It might benefit these programs to target and more carefully monitor lakes for microcystin that consistently display low secchi depth.

Applications to Future Paleolimnological Studies

Though cyanotoxin measurements in sediments are not common, some paleolimnological studies have incorporated microcystin into their measured parameters. Paleolimnological studies have been conducted on lakes in Poland, Nebraska, USA, and Canada to track eutrophication and microcystin production through time (Efting et al. 2011, Kaczorowska and Kornijow 2012, Pawlik-Skowronska et al. 2010, Zastepa et al. 2017[2]). The studies of microcystin deposition in the sediments have been limited to short (maximum of 50 cm) sediment-water interface cores that span the past ~200 years and studies of 1 to 3 lakes in an area. When compared to more established paleolimnological tools such as diatoms and photosynthetic pigment concentrations, these paleolimnological studies demonstrate the potential of sedimentary microcystin concentrations to reconstruct cyanotoxin production through time and demonstrate relationships to other established proxies such as nutrients, diatoms, and stable isotopes. Kaczorowska and Kornijow (2012) used microcystin measurements and chironomid head capsules in a sediment core from Poland to show that microcystin concentrations tracked with chironomid change. However, only one study by Zastepa et al. (2017[2]) has attempted to directly document the relationships between sedimentary microcystin concentrations and other sedimentary variables. The few studies on sedimentary microcystin show long-term production that could be compared to other sedimentary variables. This study demonstrates the potential to temporally reconstruct microcystin production, and subsequently the drivers of microcystin production, to provide key data as to when cyanotoxin occurrence began in lake systems and what limnological characteristics seem to promote cyanotoxin production.

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Tables

Table 2.1. General characteristics of study lakes.

Lake	Latitude	Longitude	Surface Area (km^2)	Mean Depth (m)	Secchi Depth (m)	Trophic Status	Total MY Concentration (ng g^{-1} org)
Annie	27.99159	-81.6063	1.80	3.68	2.3	eutrophic	6.54
Apopka	28.62873	-81.6164	124.12	1.84	0.4	hypereutrophic	5.20
Banana	27.9773	-81.9021	1.02	1.05	0.4	hypereutrophic	54.85
Bear	30.86359	-86.8309	0.44	2.10	1.1	eutrophic	6.98
Beauclair	28.77488	-81.6522	4.07	2.63	1.1	hypereutrophic	44.84
Bonnet	27.54356	-81.4411	1.05	0.95	0.8	hypereutrophic	44.65
Bonny	28.03791	-81.9269	1.08	0.79	0.6	hypereutrophic	214.53*
Carlton	28.76002	-81.6572	1.61	3.41	0.6	hypereutrophic	244.18*
Conine	28.05973	-81.7247	0.96	2.63	1.1	hypereutrophic	59.07
Crescent	29.46805	-81.5032	64.59	2.00	0.5	eutrophic	1.38
Crystal	28.02596	-81.9099	0.11	1.84	0.9	n/a[@]	66.29
Cypress	28.07814	-81.3190	16.58	1.39	0.6	hypereutrophic	30.49
Dead	29.41520	-81.4381	1.61	n/a	0.4	eutrophic	0.87
Doctors	30.13325	-81.7360	13.75	3.00	1.0	eutrophic	7.72
Dora	28.78911	-81.6844	18.22	2.40	0.6	hypereutrophic	64.20
Eustis	28.84645	-81.7241	31.59	4.46	0.8	hypereutrophic	63.97
George	29.26681	-81.5840	186.16	2.10	0.5	eutrophic	12.92
Glenada	27.56280	-81.5071	0.72	2.05	0.6	hypereutrophic	347.03*
Griffin	28.87613	-81.8430	4.07	2.90	0.8	hypereutrophic	36.30
Henry	27.32344	-81.3834	0.26	n/a	1.5	eutrophic	25.47
Hollingsworth	28.02371	-81.9449	1.44	1.05	0.6	hypereutrophic	180.74*
Hunter	28.03378	-81.9660	0.40	1.58	0.5	hypereutrophic	67.90
Hurricane	30.94106	-86.7568	1.29	1.84	2.1	eutrophic	1.53
Istokpoga	27.38594	-81.2917	121.88	1.05	0.7	eutrophic	5.54
Jackson	30.50932	-84.3070	16.20	1.84	1.8	eutrophic	1.15
Jesup	28.71977	-81.2256	40.51	1.58	0.9	hypereutrophic	123.86*
Kissimmee	27.92111	-81.2612	198.07	1.31	0.6	eutrophic	61.24
Lochloosa	29.51873	-82.1238	22.86	1.63	0.5	hypereutrophic	29.07
Maggiore	27.73694	-82.6536	1.54	1.16	0.6	hypereutrophic	94.54
Maitland	28.61894	-81.3524	1.83	n/a	2.7	eutrophic	7.53
Marian	27.87745	-81.0854	23.22	3.41	0.8	hypereutrophic	674.12*
Middle	28.42149	-82.3156	1.52	n/a	1.0	n/a	23.72
Monroe	28.84424	-81.2674	37.47	1.58	0.7	eutrophic	3.21
Orange	29.45046	-82.1696	56.09	1.44	0.7	eutrophic	49.85
Otter	30.02466	-84.4220	0.54	n/a	0.7	mesotrophic	1.62
Persimmon	27.35523	-81.4056	0.18	1.73	0.4	hypereutrophic	5.16
Pierce	27.97278	-81.5247	15.09	1.84	0.6	eutrophic	83.20
Placid	27.97278	-81.5247	12.99	5.44	1.0	eutrophic	2.84

Rowell	29.92093	-82.1578	1.47	1.30	0.5	eutrophic	51.67
Seminole	27.85228	-82.7817	2.90	n/a	0.5	hypereutrophic	48.60
Stone	30.96507	-87.2899	0.53	1.58	1.2	eutrophic	2.29
Thonotosassa	28.06013	-82.2773	3.31	1.58	0.6	hypereutrophic	57.35
Tohopekaliga	28.19484	-81.3860	76.12	2.40	1.0	eutrophic	7.29
Watertown	30.19276	-82.5999	0.19	3.80	1.3	eutrophic	8.97
Wauberg	29.52947	-82.3012	1.50	3.15	0.6	hypereutrophic	26.59
Weohyakapka	27.82509	-81.4172	29.64	2.10	0.8	eutrophic	0.32
Yale	28.91135	-81.7330	16.36	3.70	1.1	eutrophic	16.66

[@]Values listed as n/a were not recorded or described in the Lakewatch database. *Indicates MY concentrations values that fell above ELISA kit detection limit and thus were determined using an extended curve of-best-fit.

Table 2.2. Summary statistics of variables measured in all study lakes (n=47).

Parameter	Mean	Median	Maximum	Minimum	Standard Deviation	r^2 with Total MY Concentration
Surface Area (km^2)	24.66	2.90	198.07	0.11	45.80	0.0106
Mean Depth (m)	2.18	1.84	5.44	0.79	1.03	0.0038
Secchi Depth (m)	0.9	0.7	2.7	0.4	0.5	0.0377
Canthaxanthin (nmol g^{-1} org)	26.09	20.11	112.78	0.00	23.47	0.0022
Aphanizophyll (nmol g^{-1} org)	20.86	17.25	119.67	0.00	24.09	0.0415
Chlorophyll-a (nmol g^{-1} org)	38.14	33.32	148.14	0.75	26.34	0.0305
TOC (%)	25.06	25.82	41.56	0.61	10.13	0.0734
TN (%)	2.35*	2.36	5.32	0.06	1.13	0.1243
P (mg/g)	1.88	1.62	6.58	0.11	1.15	0.0686
Fe (%)	9.74	7.50	26.85	0.61	6.82	0.0105
S (%)	9.97	7.50	26.98	0.21	6.63	0.0068
TOC:TN	13.26	12.47	32.19	7.79	3.73	0.0568
TN:P	31.94	29.54	87.72	8.62	17.04	0.0003
Fe:P	38.00*	31.58	145.46	4.61	30.96	0.0910
Total MY Conc. (ng g^{-1} org)	63.28	29.07	674.12	0.32	114.62	—

Table 2.3. Summary statistics of variables measured in "high" lakes classified as MY-outliers (n=6)

Parameter	Mean	Median	Maximum	Minimum	Standard Deviation	r^2 with Total MY Concentration
Surface Area (km^2)	26.59 (11.43)	3.31 (1.52)	198.07 (40.51)	0.11 (0.72)	48.44 (16.75)	0.0089 (0.0072)
Mean Depth (m)	2.20 (2.05)	1.84 (1.81)	5.44 (3.41)	0.95 (0.79)	1.03 (1.14)	0.0537 (0.4288)
Secchi Depth (m)	0.90 (0.68)*	0.70 (0.60)	2.70 (0.94)	0.40 (0.57)	0.50 (0.14)	0.1224 (0.0008)
Canthaxanthin (nmol g^{-1} org)	26.22 (25.25)	19.79 (25.79)	112.78 (60.67)	0.00 (0.00)	23.77 (23.37)	0.0050 (0.0523)
Aphanizophyll (nmol g^{-1} org)	19.20 (32.18)	12.08 (30.62)	119.678 (60.01)	0.00 (15.76)	24.79 (15.69)	0.0874 (0.0005)
Chlorophyll-a (nmol g^{-1} org)	36.90 (46.57)	30.30 (47.74)	148.15 (69.62)	0.75 (27.39)	27.50 (15.37)	0.0927 (0.0105)
TOC (%)	24.41 (29.50)	24.30 (29.40)	41.56 (37.55)	0.61 (21.13)	10.47 (6.32)	0.0927 (0.2595)
TN (%)	2.26 (2.99)	2.25 (2.95)	5.32 (3.76)	0.06 (2.24)	1.15 (0.67)	0.3402 (0.2540)
P (mg g^{-1})	1.81 (2.33)	1.51 (2.27)	6.58 (3.09)	0.11 (1.69)	1.20 (0.58)	0.2374 (0.1495)
Fe (%)	10.26 (6.24)	7.64 (3.49)	26.85 (15.25)	0.61 (1.46)	6.90 (5.51)	0.0234 (0.3209)
S (%)	10.13 (8.89)	9.20 (6.98)	26.98 (15.47)	0.21 (4.47)	6.90 (4.65)	0.0139 (0.1932)
TOC:TN	13.49 (11.68)*	13.08 (11.64)	32.19 (13.28)	7.79 (10.45)	3.93 (1.03)	0.2244 (0.0127)
TN:P	32.28 (29.62)	29.54 (28.03)	87.72 (47.35)	8.62 (14.63)	17.83 (11.00)	0.0043 (0.0060)
Fe:P	41.34 (15.17)*	33.17 (7.78)	145.46 (35.52)	5.32 (4.61)	31.46 (13.47)	0.2210 (0.0582)
Total MY Conc. (ng g^{-1} org)	29.01 (297.41)	23.72 (229.35)	94.54 (674.12)	0.32 (123.86)	27.17 (198.90)	———

*Indicates that variable mean of "high" lakes (n=6) is statistically significantly different from the variable mean of "low" lakes (n=41). Values outside of parentheses represent "high" lakes and values within parentheses represent "low" lakes.

Figures

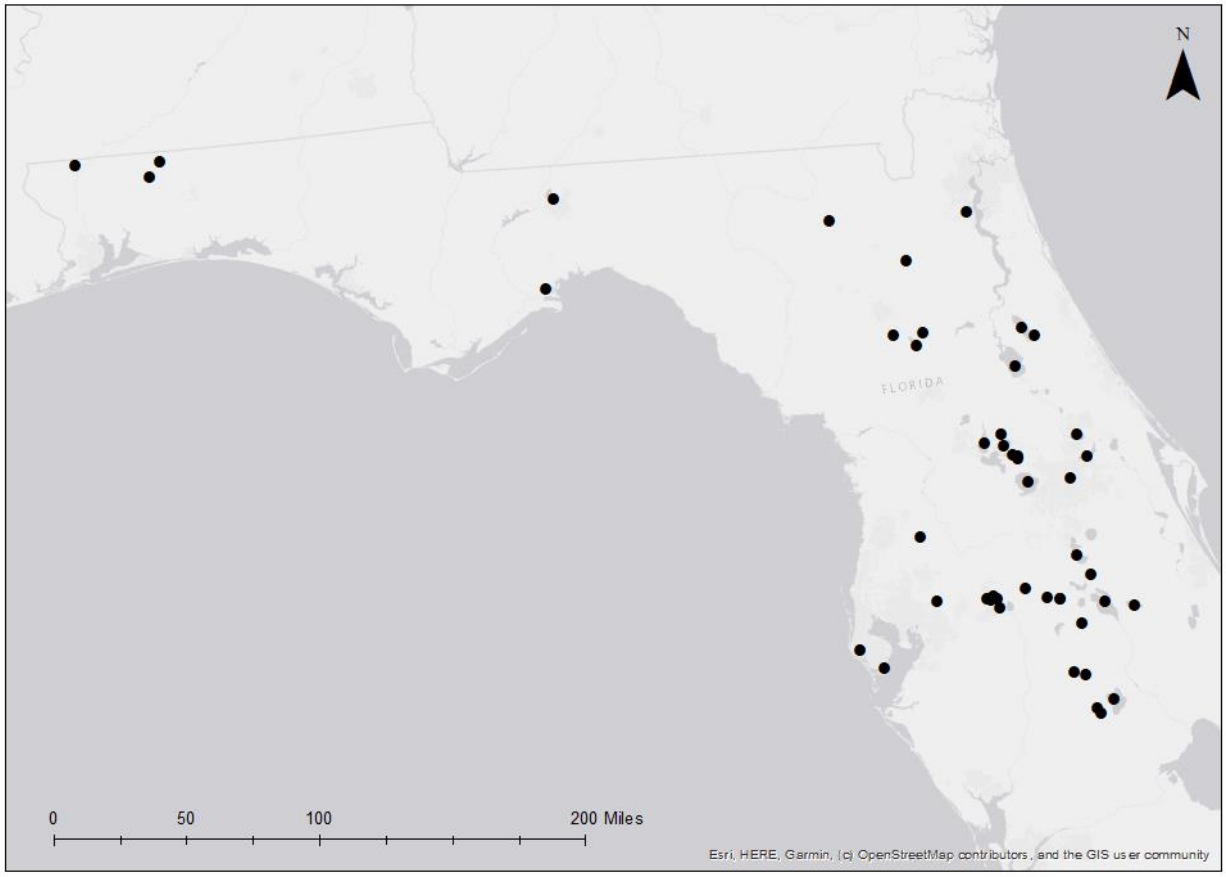

Figure 2.1. Map of 47 lakes sampled in Florida, USA.

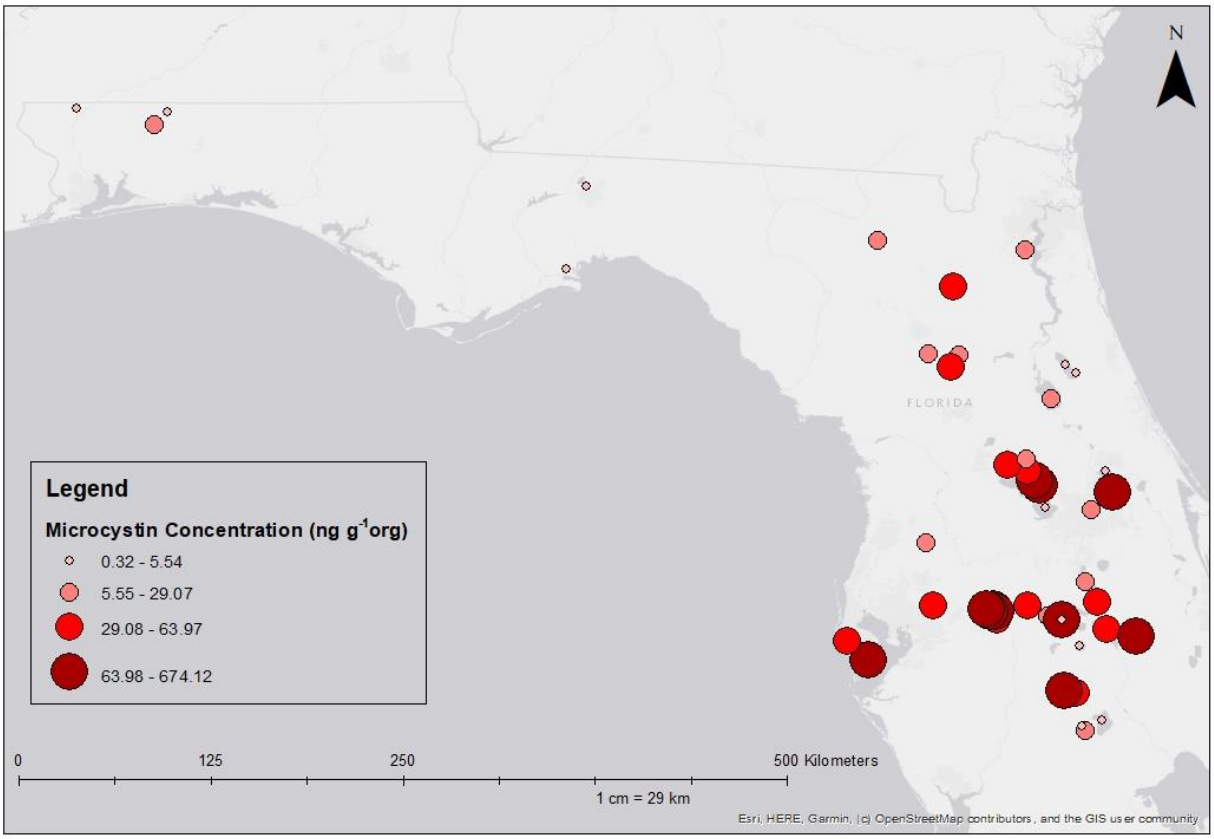

Figure 2.2. Heat map of microcystin concentrations in 47 Florida lakes.

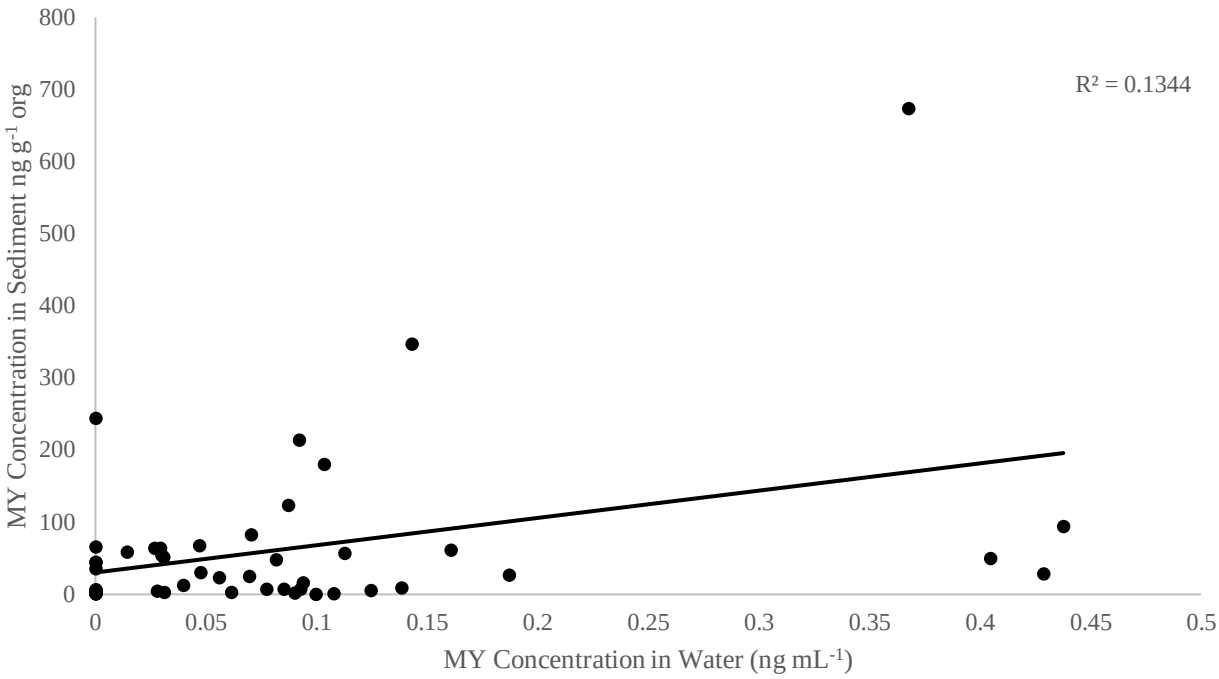

Figure 2.3. Correlation between microcystin concentration in the water column and in the surface sediment.

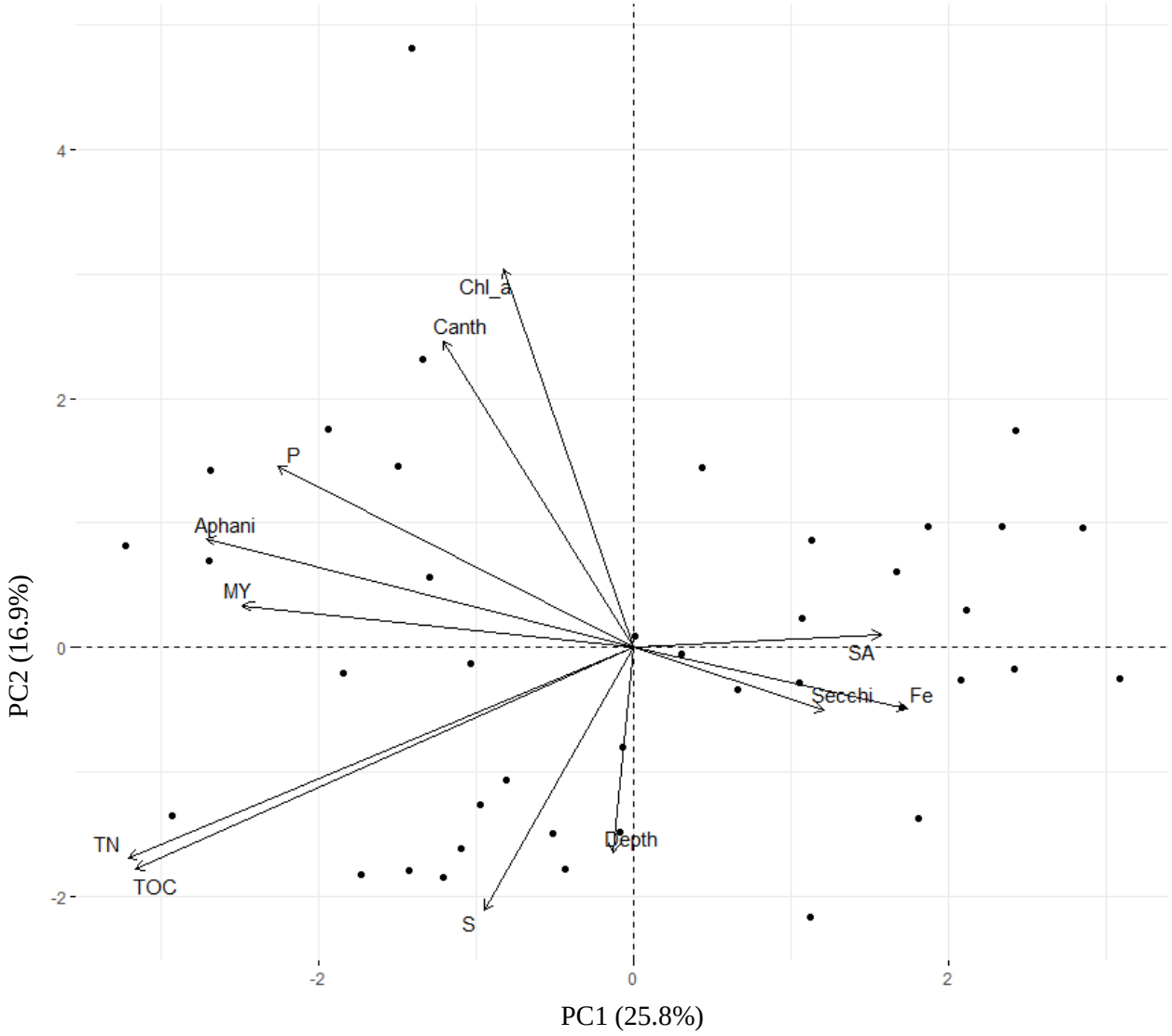

Figure 2.4. Principal component analysis (PCA) of nutrient, biological, and morphological variables that are hypothesized trigger of microcystin production in all study lakes (n=47). Aphani is aphanizophyll; Canth is canthaxanthin; Chl-a is chlorophyll-a; P is phosphorus; TN is nitrogen; TOC is total organic carbon; S is sulfur; Fe is iron; Depth is mean depth; Secchi is secchi depth; SA is surface area; MY is microcystin concentration in sediment;

Chapter 3: Characterizing Temporal Change in Nutrient Deposition in Florida Lakes

Abstract: During the last two decades, eutrophication in lakes has increased throughout the world. Legislation and regulation of external nutrient inputs into water bodies have been enacted in an attempt to mitigate increases in trophic status. However, it is unclear whether management attempts to minimize nutrient inputs into water systems have been effectual in decreasing aquatic nutrient concentrations to pre-disturbance levels. This research analyzed sedimentary variables (organic matter, total carbon, total nitrogen, total phosphorus, molar carbon:nitrogen ratios, and molar nitrogen:phosphorus ratios) and water column variables (total nitrogen, total phosphorus, and secchi depth) in sediment samples from 18 Florida lakes. Sediment core tops (modern) and bottoms (~150 years ago) were combined with a surface sediment data set from the 1980s to answer two primary research objectives: 1) to determine if the greatest period of nutrient depositional change occurred pre- or post-1980 and 2) to identify specific nutrients or nutrient stoichiometries that showed the greatest depositional and water column change. Results show that sedimentary organic matter and total carbon did not significantly change over time, though C:N ratios significantly decreased post 1980 indicating a shift towards a more phytoplankton-dominated primary producer community. Total nitrogen and phosphorus in the sediment have both significantly increased over time, and N:P ratios have significantly decreased potentially favoring N-fixing cyanobacteria. Water column total nitrogen and phosphorus significantly decreased in the past 40 years, while secchi depth (light attenuation) remained stable. The differences between sedimentary and water column nutrient trends suggest that even though external nutrient inputs have decreased, nutrient concentrations in the sediment could be internally loaded and sustaining phytoplankton populations through resuspension events. These 18 study lakes appear to have gone through much less limnological change in the past 40 years

when compared to the 100 years prior, suggesting that substantial ecological shifts might have preceded monitoring data.

Introduction

During the last two decades lake eutrophication and cyanobacteria dominance has increased throughout the world (Ewing et al. 2020, Paerl and Huisman 2008, Taranu et al. 2015), and concern over increases in harmful algal blooms are especially strong in waterbodies that are utilized for anthropogenic purposes (Bullerjahn et al. 2016, Carmichael and Boyer 2016). Widespread consensus of increasing intensity and widening geographic distribution of nutrient inputs and algal blooms are generally attributed to increased temperatures (via climate change) and anthropogenic eutrophication (Paerl and Huisman 2008, Taranu et al. 2015). Both federal and state-sanctioned legislation and regulation of external nutrient inputs into water bodies have been enacted in an attempt to mitigate these increases in algal bloom policies established in the last 30 to 40 years (EPA, Hudnell 2010). However, it is unclear whether efforts to minimize nutrient inputs into water systems have been effectual in decreasing aquatic nutrient concentrations (Dove and Chopra 2015).

With increased concern over anthropogenic-derived nutrient transport into water bodies and cyanobacteria blooms, measurements of nutrients and algal abundance are frequently integrated into water monitoring programs (EPA National Lakes Assessment, Florida LAKEWATCH). However, little is known of the prevalence of nutrient dynamics and cyanobacteria blooms prior to these monitoring efforts. Because measurements are expensive, monitoring frequencies are typically limited to intermittent samplings (monthly, annually) of a few locations. Primary producers in aquatic systems respond quickly to environmental changes like alterations in temperature, rainfall, wind, and other environmental variables. Thus, environmental variability

confounds the representative nature of infrequent sampling and can complicate monitoring efforts and the ability to identify limnologic trends. However, sediment analysis and the application of paleolimnological tools can incorporate longer timescales and more broad-scale and whole-ecosystem analysis than prolonged water sampling (Smol 2008). Paleolimnology utilizes sediment deposition as a long-term dataset representative of whole-lake environmental conditions. Analysis of sediment cores can produce data over longer temporal scales (i.e. annual, sub-decadal, decadal periods) thus preceding most monitoring efforts on the most studied lake systems. Sediment deposition integrates yearly samples related to long-term processes instead of daily environmental fluctuations (Binford and Brenner 1988).

Due to its high lake density, prevalence of eutrophic conditions and a relatively long-term monitoring program (LAKEWATCH, ~35 years), the state of Florida, USA offers a unique perspective of nutrient dynamics and eutrophication comparisons over time. Because of these natural characteristics and anthropogenic inputs of nutrients into aquatic bodies, Florida lakes are experiencing increased eutrophication at a noticeable rate (Riedinger-Whitmore et al. 2005). Florida lakes are subtropical and shallow, conditions that encourage polymixis and discourage thermal stratification (Brenner et al. 1990). Warm temperatures, mixing that promotes internal nutrient loading, and long residence times result in extended growing seasons from primary producers and multiple biogeochemical pathways for nutrient dynamics. This research analyzed trends in sedimentary variables (organic matter, total carbon, total nitrogen, total phosphorus, molar carbon:nitrogen ratios, and molar nitrogen:phosphorus ratios) and water column variables (total nitrogen, total phosphorus, and secchi depth) in three sediment samplings that represent the previous ~150 years of limnologic nutrient dynamics in 18 Florida lakes. The two primary research objectives were: 1) to determine if the greatest period of nutrient depositional change

occurred pre- or post-1980 and 2) to identify specific nutrients or nutrient stoichiometries that showed the greatest depositional and water column change.

Methods

The eighteen sample lakes chosen for this study are located within the peninsula of Florida, USA (Figure 3.1). Selection of lakes was based upon overlap between two larger surface sediment and water sample studies, my forty-seven lakes dataset and a 1980s survey conducted by Binford and Brenner (1988). Lake physical characteristics, including location, surface area, mean depth, and trophic state show the variety of lake systems studied (Table 3.1) (LAKEWATCH).

Modern and Historic Samples

Sediment and water samples were collected during the summer of 2018. Sediment cores were collected using a piston-coring device that maintains an undisturbed sediment-water interface (Fisher et al. 1992). The top 4 cm of the sediment core were immediately sectioned and designated as the "surface sediment" sample. ^{210}Pb dates from previous sediment core studies of Florida lakes indicate that the surface sediment samples are representative of the previous 2-10 years of sediment deposition (Brenner and Binford 1988). Collecting the top 4 cm of sediment as opposed to a smaller section was used based on previous paleolimnological investigations in Florida (Brenner et al. 1999, Schelske et al. 2005, Waters et al. 2015) and to allow for enough material for sediment analytical measurements. Surface sediments for many Florida lakes are flocculent so the sample section of 4-cm also accounted for material that can be resuspended due to short-term environmental fluctuations (e.g. wind and fetch) in shallow basins (Scheffer and van Nes 2007). The surface sediment samples are therefore designated as "modern" samples for the remainder of the study.

The bottom-most 4 cm of the sediment cores were also collected. With a 120-cm coring barrel and allowing for 20-30 cm of clearance for the water-sediment interface, we estimate an average sediment core length of ~80 cm, unless sediment accumulation at the coring limited the length of the collected core to less material. The deepest lake sediments (not underlying soil or marl) in the core were saved to represent a period of pre-human disturbance. The use of sediment core bottoms to represent a period prior to human disturbance is not novel and was previously used to estimate the extent of anthropogenic impact to other water bodies (Bachmann et al. 2013, Bennion et al. 2015, Chen et al. 2011).

Paleolimnological studies of Florida lakes frequently utilize ^{210}Pb to provide a corresponding dating record. Gamma spectrometry measurements of radioisotope activities in the sediments are collected via well-type germanium detectors according to methods of Appleby et al. (1986) and Schelske et al. (1994). The depth to reach supported ^{210}Pb activity is indicative of the temporal limit of the dating method. The depth at which unsupported ^{210}Pb concentrations reach zero is approximately 8.5 half-lives of the radioisotope. Eight and half half-lives is equated (with conservation) to ~150 years. Thus, the depth of unsupported ^{210}Pb in a sediment core indicates ~150 years before collection, prior to human impacts. The depth at which unsupported ^{210}Pb reaches zero in 32 previously dated Florida sediment cores is listed in Table 3.2. If multiple sediment cores or sediment studies published different depths, the deepest, most conservative value is listed. The average depth to reach the limit of unsupported ^{210}Pb is 55 cm, with only 5 lakes having a depth greater than or equal to 80 cm, but not exceeding 85 cm. Because we estimate an average sediment core length of ~80 cm—well below the 55 cm metanalytical mean—we are confident that the bottom sediment samples are indeed indicative of pre-

anthropogenic activities and impacts. The bottom sediment samples are therefore termed "historic".

Three sediment cores (i.e. three modern samples and three historic samples) were collected from each lake as replicates. The three sampling locations within each lake were largely selected based on prior knowledge of the surface sediment distribution and available bathymetric maps (see Chapter 2). If prior work had not documented sediment distributions, sampling locations were then chosen based upon the location with the highest likelihood of organic matter accumulation in the lake. Surface sediment samples were stored on ice and in the dark until they were returned to the laboratory at Auburn University. A wet aliquot of known volume was removed for bulk density and determination of organic content. The remaining samples were frozen, freeze-dried, and mechanically ground with a mortar and pestle for analysis. Freeze dried sediment samples were then analyzed for total carbon (TC), total nitrogen (TN), and total phosphorus (TP).

From each study lake, water samples were collected using an integrated water sampler. Water samples were collected from the most centrally-located sediment coring site and were filtered within 24 hours of collection. The water filtrate was refrigerated for the analysis of TC, TN, and TP. Secchi depth for each lake were determined at the same location as water sampling.

Organic matter content of the sediment samples was calculated by loss-on-ignition (LOI) at 550°C in a muffle furnace for 3 hours and expressed as a percent (Hakanson and Jansson 1983). TC and TN on the freeze-dried sediment were measured using a combustion CHN Costech Elemental Combustion System. TP (in freeze dried sediment and in sample water filtrate) and TN (in the filtered water) were measured by ion coupled plasma (ICP) following the EPA Method 6010B. TN in the water samples is a sum of ammonia-N and nitrate-N.

Mid Samples

Surface sediment and water samples were analyzed for the same 18 study lakes in a 1982 study using similar methods (Brenner and Binford 1988). Collected surface sediments were the top two centimeters of undisturbed, surficial grabs from an Eckman dredge. Organic matter content was determined by loss on ignition at 550°C. TC was measured with a LECO induction furnace, TN measured via modified Kjeldahl digestion, and TP concentrations determined spectrophotometrically (Brenner and Binford 1988). Methods of water variables are not reported. Samples collected in 1982 are thus termed "mid" as they temporally fall between my study's "modern" and "historic" samples. Thus, the combined dataset of the historic, mid, and modern sediment variables forms a ~150 dataset of nutrient deposition in the 18 study lakes, and the combined water samples of my study and the Brenner and Binford (1988) study form a water column analysis spanning ~40 years.

Statistical Analysis

To determine significant differences between modern, mid, and historic sediment samples, one-way analysis of variance (ANOVA) tests were conducted for all variable comparisons. If ANOVA results indicated differences between the groups, t-tests were performed to elucidate the specific differences. For the water column samples, the modern water samples and the mid water samples were statistically compared by t-tests to determine significant differences. All statistics were completed in R (R Core Team 2020).

Results

Mean organic matter content (LOI) in the sediment samples increased over time from 38.0 +/- 23.4% (historic) to 40.1 +/- 22.6% (mid) to 49.8 +/- 10.5% (modern) but the changes in LOI between temporal groups were not significantly different ($p=0.17$) (Table 3.3, Figure 3.2).

Concentrations of total carbon (TC) displayed less of a consistent trend, initially decreasing between historic (22.2 +/- 12.2%) and mid (20.7 +/-11.7%) and then increasing in modern (25.8 +/- 11.0%) samples (Table 3.3, Figure 3.3). Mean TC concentrations between historic, mid, and modern were also not significantly different ($p=0.41$) (Figure 3.3). TC represents a proportion of the organic matter (LOI%) fraction in historic (64 +/- 20%), mid (53 +/- 5%), and modern (55 +/- 28%) samples. The stoichiometric ratio of molar TC to molar TN (C:N) shows a temporally decreasing pattern from historic to modern. C:N values decrease from 22.0 +/- 9.5 in the historic samples to 12.0 +/- 2.5 and 12.5 +/- 2.1 in the mid and modern samples, respectively (Table 3.3). ANOVA analysis and t-tests indicate that C:N in historic samples are significantly less than mid and modern samples ($p<0.0005$)(Figure 3.4).

Total nitrogen (TN) and total phosphorus (TP) in the sediment samples show similar increasing trends over time. Mean sediment TN concentrations increased from 1.4 +/- 0.9% to 2.0 +/- 1.1% to 2.4 +/- 1.1% in historic, mid, and modern samples, respectively (Table 3.3). Historic samples of TN were significantly less than mid and modern samples ($p=0.01$) (Figure 3.5). Mean sediment TP concentrations increased from 0.8 +/- 0.8 mg g^{-1} in historic samples to 2.0 +/- 2.3 mg g^{-1} and 1.6 +/- 0.7 mg g^{-1} in the mid and modern samples (Table 3.3). Though mean sediment TP decreased slightly from mid to modern, the values are not significantly different. However, historic TP is significantly less than both mid and modern TP ($p=0.04$) (Figure 3.6). Stoichiometric molar ratios of TN and TP (N:P) significantly decreased over time. Historic samples had a N:P ratio of 72.3 +/- 66.7, decreasing to 33.9 +/- 23.4 in mid and 36.9 +/- 16.4 in modern samples (Table 3.3). Again, N:P in historic samples are significantly different than N:P in the mid and modern samples ($p=0.01$) (Figure 3.7).

Analyses between mid and modern water samples showed that mean secchi depth between the mid (0.86 +/- 0.52 m) and modern (0.88 +/- 0.52 m) water samples increased slightly but were not significantly different (p=0.47) (Table 3.3, Figure 3.8). Both mean TN and TP concentrations in the water significantly decreased between the mid samples (TN = 1.61 +/- 0.90 mg L^{-1}, TP = 0.142 +/- 0.172 mg L^{-1}) and the modern samples (TN = 0.55 +/- 0.49 mg L^{-1}, TP = 0.036 +/- 0.079 mg L^{-1}) (p_{TN}<0.0005, p_{TP}=0.01)(Table 3.3, Figure 3.9, Figure 3.10).

Data collated from LAKEWATCH's long term trends of water column TP, TN and secchi depth in our study lakes is displayed in Figure 3.4 (LAKEWATCH). Long-term data and trends are not available for three of my lakes (Maitland, Marian, Watertown). LAKEWATCH has plotted all of the available measurements for the variables and evaluated the data from trends with regression analyses. Significant trends have a p-value < 0.05. Of the data available for 15 lakes, 8 lakes have no discernible TP trends, 4 lakes have increasing TP, and 3 lakes have decreasing TP. Eleven of 15 lakes have no TN trends, 3 lakes have increasing TN, and one lake shows a decreasing TN trend. Of the secchi depth measurements in the 15 available data sets, 7 lakes have neither increasing or decreasing trends, 2 lakes are increasing in secchi depth, and 6 lakes have decreasing secchi depth.

Discussion

Both sedimentary organic matter (LOI) and TC values show slightly increasing trends over time, but neither variable demonstrates significant differences between historic, mid, and modern samples. Organic matter and organic carbon delivered to the sediments in these 18 study lakes have maintained similar amounts throughout the last ~150 years. Organic matter is generally high, with means ranging from 38.0% - 49.8%, due to relatively small watersheds and little organic input from the surrounding landscape (Wetzel 2001). However, just because the

amount of organic matter and TC in the sediments is not significantly changing does not necessarily mean that the source of carbon (allochthonous, autochthonous) also remains the same (Meyers and Teranes 2001).

Stoichiometric ratios of total carbon : total nitrogen (C:N) can indicate the source of organic matter—phytoplankton (autochthonous), macrophytes (autochthonous), or land plants (allochthonous)—due to differences in concentrations of cellulose and lignin (Meyers et al. 1993). Land plants typically have C:N ratios greater than 20, aquatic plants typically between 4 and 10, and algae less than 5 (Meyers et al. 1993). Sedimentary C:N ratios decreased from 22.0 (historic) to 12.0 and 12.5 (mid and modern, respectively). Historic C:N is significantly higher than mid and modern values ($p<0.0005$), indicating a substantial shift towards greater dominance of macrophytes and algae/cyanobacteria in primary producer community prior to the 1980s. Dramatic shifts in primary producer community structure have been documented in prior paleolimnological studies of Florida lakes (Kenney et al. 2002, Schelske et al. 2005, Waters et al. 2005). Current dominance of macrophytes and phytoplankton is also supported by previous studies of Florida lakes that show the majority of organic matter currently in lake systems is derived from macrophytes and algal productivity (autochthonous sources) instead of terrestrial inputs (allochthonous sources) (Brenner et al. 1999b, Waters et al. 2019).

While the temporal alterations of organic carbon storage suggest autochthonous origin, shallow lakes (like my study lakes) have been shown to alternate between stable ecological states dominated by macrophytes or algae/cyanobacteria (Scheffer et al. 2003). Stable dominant states of macrophytes and algae can coexist in the same lake system over larger temporal scales (years, decades, centuries) (Schelske et al. 2005). Alternative stable states in shallow lakes, either large or small, are dependent upon strong reinforcing positive feedbacks (Scheffer 2007). The same

possible conditions (e.g. nutrient loading, turbidity, fetch, etc.) can reinforce the conditions of either a clear, macrophyte state or a turbid, phytoplankton state (Janssen et al. 2014). Transitions between alterative stable states in Florida ecosystems have been documented in multiple paleolimnological studies (Waters et al. 2015, Kenney et al. 2002, Schelske et al. 2005). For example, Lake Apopka, FL is a shallow, hypereutrophic lake that abruptly changed from a macrophyte-dominant state to a phytoplankton-dominant state in 1947. Though Lake Apopka was dominated by macrophytes during most of its Holocene history (Waters et al. 2015), its phytoplankton-dominant state has persisted despite management efforts to restore macrophyte habitation. Even though Lake Apopka has undergone a dramatic shift from macrophytes to algae (thus affecting its C:N ratios), its concentrations of sedimentary carbon have remained within 10% of itself for the past 8,000 years (Waters et al. 2015) thus supporting the similar temporal TC storage in my study lakes. Multi-lake investigations of organic matter and carbon in Florida sediments also support the persistence of C through substantial ecological change and further suggest that there was more of a macrophyte signal in Florida lakes in the past (Kenney et al. 2002, Waters et al. 2019a). Thus, a lack of significant change in organic matter and carbon between historic, mid, and modern samples does not negate the possibility that decreasing C:N ratios document a shift towards algal dominance in this 18-lake study.

Sedimentary TN concentrations significantly increased between historic samples and mid and modern samples, but there was no significant increase between mid and modern TN values. Increases in TN input into water bodies has been linked to increases in synthetic nitrogen fertilizers and the cultivation of nitrogen-fixing crops, which began around the mid-1800s in Florida (Battye et al. 2017, Cao et al. 2018). Nitrogen burial in the sediment does not typically result in long term storage, and because of its atmospheric component, the representation of

measured sediment TN can be skewed depending on other external factors, including rates of microbial transformation that are not typically measured in sediment (Finlay et al. 2013). TN concentrations in the sediment, though notably increasing prior to the 1980s in these 18 lakes, might not reflect the water column conditions as much as TC or TP.

Sedimentary TP displays a similar significant increase to that of sedimentary TN. TP concentrations are significantly higher in the mid and modern samples than in historic samples indicating that the period of greatest P inputs occurred prior to 1982 and thus most monitoring efforts. Sedimentary TP has increased over time (in reference to historic values) but has not increased in the past 40 years. The apparent consistency of TP concentrations in the sediment between mid and modern samples could be attributed to increased regulations and implemented total maximum daily loads (TMDLs) on both federal and state levels (FDEP). Though TP inputs seem to have decreased, or at a minimum, stabilized, environmental P cycling includes no atmospheric component, and thus the P introduced and present in the waterbodies remains until it is bioassimilated by algae, bacteria, and other primary producers, transported aqueously out of system, or bound and deposited into the sediments (Torres et al. 2014, Wetzel 2001). In the shallow lakes of Florida, large pools of sedimentary-P can be reintroduced into the water column by fetch-induced resuspension (Cyr et al. 2009). This results in a near-constant resupply of P into the water column that can promote cyanobacteria abundance (Moore et al. 1998). The internal loading of TP into the water column from resuspended sediments could be sustaining bioavailable P to phytoplankton even though anthropogenic inputs into the system have decreased, which is also supported by the decreasing C:N values. Lake Apopka is again an example of sustained algal/cyanobacteria biomass even though P-inputs to the system have decreased (Waters et al. 2015). Jeppesen et al. (2005) found that the effects of declines in

external TP loading (i.e. re-oligotrophication) took 10-15 years to reach a new equilibrium in the water column, suggesting effects of decreased external TP loads in the sediments could take even longer. Increased TP prior to the 1980s and sustained bioavailable P because of internal loading in the system could support continued phytoplankton abundance and account for the decreased C:N ratios since the historic samples (Brenner et al. 1999a, Kenney et al. 2002, Schelske et al. 2005).

Stoichiometric molar ratios of TN and TP (N:P) have significantly decreased between historic sediments (72.3) and mid (33.9) and modern (36.9) sediments. There is no significant change in N:P ratios between mid and modern. Temporally lower N:P indicates a movement towards lower TN relative to TP in the lake systems. N:P ratios influence competitive interactions among phytoplankton species, and ecological resource theory suggests low N:P ratios favor cyanobacteria dominance because some cyanobacteria genera can fix nitrogen and outcompete other primary producers (Dolman et al. 2012, Smith 1984). The lowering of N:P ratios in these 18 Florida lakes further supports that shifts towards phytoplankton dominance occurred prior to the 1980s.

Water column TN and TP concentrations are significantly higher in mid measurements than in modern measurements. Both TN and TP in the water column, indicators of eutrophication in water bodies, have improved (decreased) since the 1980s. However, secchi depth, a measure of light attenuation often stemming from a mixture of suspended solids, dissolved organic matter, and phytoplankton in the water column, has not significantly changed between mid and modern measurements (Swift et al. 2006). Even though water column nutrients have decreased, there is no apparent change in light attenuation, which is also supported by decreasing TC:TN and similar nutrient deposition in the sediments. It is of note that these trends are based upon

single water sample grabs from each of the 18 lakes in this study—once in the 1980s and once in 2018. LAKEWATCH, a citizen volunteer lake monitoring program, publishes reports in which they have analyzed long-term (>10 samples) trends for TN, TP, and secchi depth from annual water samples—again, single yearly samples (LAKEWATCH). LAKEWATCH was established in 1986 and therefore its most historic data loosely corresponds to our mid samples and continues for the past 35 years. In summary, of the fifteen lakes of which LAKEWATCH has long-term data, there is no consensus as to if TN, TP, and secchi depth in the 18 lakes in my study are increasing or decreasing over time (Table 3.4). A notable number of lakes (n=11) have no trends for at least one of the measured variables.

The trends of decreasing TN and TP in the water column do not necessarily contradict the lack of significant difference within the sediment TN and TP concentrations. TN and TP in water samples reflect dissolved concentrations in the water column, but sediments are focused on particulate matter (and the associated particulate nutrients) that are deposited. Water column concentrations could be showing improvement (decreased external loading) from regulations that have not become apparent in the sediment timescales (5-10 year running means) (Brenner and Binford 1988). Furthermore, water sampling is typically conducted on the most meteorologically calm days to allow for ease of sampling. “Calm” days often have reduced wind gusts and therefore induce less sediment resuspension. Nutrient-rich sediment resuspension can favor increased algal and cyanobacteria abundance, and higher cell densities can decrease secchi depth measurements (Mihaljević and Stević, 2011). Thus, sampling on days with less resuspension to the water column might underestimate the TN and TP in flux between the sediment and water column that sustains algal and cyanobacteria populations.

Combining the LAKEWATCH trends and our water TN, TP, and secchi depth comparisons with our sedimentary evidence of substantial change occurring prior to the 1980s indicates that monitoring data—even 35 years of measurements—may not be adequately capturing the period of largest nutrient change within these aquatic ecosystems. It is widely acknowledged that algal blooms are increasing in intensity and frequency despite increases in research, monitoring, and management behaviors (Paerl and Huisman 2008, Teranu et al. 2015). However, a recent 650-lake analysis from 30-year monitoring data suggests that algal bloom intensification might not be as pervasive as currently believed, as 67% percent of the study lakes did not exhibit increasing or decreasing trends in summertime algal bloom intensity (derived from chlorophyll concentrations, nutrient concentrations, secchi depth measurements, and climate data) (Fernandez-Figueroa 2021). It is possible that limited monitoring records (~30 years) may not sufficiently capture nutrient, eutrophication, and algal bloom trends suggesting the need to couple these monitoring data with paleolimnological studies to establish preexisting conditions and to set management targets.

Conclusions

Environmental variability confounds the representative nature of infrequent sampling and can complicate monitoring efforts. Timescales are crucial in understanding the eutrophication and management of lake systems. The measurement of variables in the sediment record can incorporate longer timescales and more broad-scale and whole-ecosystem analysis of nutrient dynamics and cyanobacteria proliferation. Based upon measured sediment parameters, this 18-lake study set appears to have gone through much less limnological change in the past 40 years compared to the ~110 years prior. These trends are not apparent when limited to recent water-monitoring data, and thus sediments (and paleolimnology) offer a more in-depth perspective of

long-term limnologic trends. It would benefit monitoring program perspectives to include that large scale ecological change could have preceded efforts, confounding interpretation and management courses for the future of these lake systems.

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Tables

Table 3.1. Physical characteristics of the 18 study lakes, including location (latitude and longitude), surface area, mean depth, and trophic status according to LAKEWATCH classification.

Lake	Latitude	Longitude	Surface Area (km^2)	Mean Depth (m)	Trophic Status
Apopka	28.62873	-81.6164	124.12	1.84	hypereutrophic
Cypress	28.07814	-81.3190	16.58	1.39	hypereutrophic
Doctors	30.13325	-81.7360	13.75	3.00	eutrophic
Eustis	28.84645	-81.7241	31.59	4.46	hypereutrophic
Glenada	27.56280	-81.5071	0.72	2.05	hypereutrophic
Griffin	28.87613	-81.8430	4.07	2.90	hypereutrophic
Istokpoga	27.38594	-81.2917	121.88	1.05	eutrophic
Jesup	28.71977	-81.2256	40.51	1.58	hypereutrophic
Kissimmee	27.92111	-81.2612	198.07	1.31	eutrophic
Lochloosa	29.51873	-82.1238	22.86	1.63	hypereutrophic
Maitland	28.61894	-81.3524	1.83	n/a	eutrophic
Marian	27.87745	-81.0854	23.22	3.41	hypereutrophic
Orange	29.45046	-82.1696	56.09	1.44	eutrophic
Thonotosassa	28.06013	-82.2773	3.31	1.58	hypereutrophic
Tohopekaliga	28.19484	-81.3860	76.12	2.40	eutrophic
Watertown	30.19276	-82.5999	0.19	3.80	eutrophic
Weohyakapka	27.82509	-81.4172	29.64	2.10	eutrophic
Yale	28.91135	-81.7330	16.36	3.70	eutrophic

Table 3.2. Summary of 32 sediment core depths at which levels of unsupported ^{210}Pb reach zero, indicating the extent of the ^{210}Pb-dating record (~150 years). All listed lakes are from previous paleolimnological studies.

Lake	Sediment Depth of Unsupported ^{210}Pb (cm)	Source
Annie	38	Quillen et al. 2011
Blue Cypress	54	Brenner et al. 2004
Clear	45	Brenner et al. 1999[2]
Conine	75	Riedinger-Whitmore et al. 2005
Dora	85	Whitmore et al. 2018
Dosson	50	Brenner et al. 2004
Griffin	38	Brenner et al. 1999[2]
Haines	50	Whitmore et al. 2018
Halfmoon	35	Brenner et al. 2004
Harris	44	Arnold et al. 2018
Hell 'n' Blazes	78	Brenner et al. 2004
Hollingsworth	80	Brenner et al. 2004
Howard	55	Brenner et al. 2004
Jesup	62	Kenney et al. 2002
Little Jackson	70	Whitmore et al. 2008
Long Pond	28	Earley et al. 2017
Lucerne	36	Brenner et al. 2004
Mariana	70	Brenner et al. 2004
May	70	Whitmore et al. 2018
Newnans	65	Brenner et al. 2004
Ocean Pond	22	Waters et al. 2019
Orange	80	Brenner et al. 1999[3]
Panasoffkee	33	Brenner et al. 2004
Parker	56	Brenner et al. 1993
Persimmon	55	Brenner et al. 2004
Sawgrass	60	Brenner et al. 1999[1]
Sheeler	28	Arnold et al. 2019
Thonotosassa	84	Brenner et al. 1996
Washington	52	Brenner et al. 1999[1]
Wauberg	85	Riedinger-Whitmore et al. 2005
Weir	30	Kenney et al. 2010
Yale	35	Kenney et al. 2010

Table 3.3. Summary of mean sediment organic content (LOI), TC, TN, TP, C:N, and N:P in modern, mid, and historic sediment samples and mean water secchi depth, TN, and TP in modern and mid water samples.

	Sediment						Water		
	LOI (%)	**TC** (%)	**TN** (%)	**TP** (mg g^{-1} org)	**C:N**	**N:P**	**Secchi** (m)	**TN** (mg L^{-1})	**TP** (mg L-1)
Modern	49.8	25.8	2.4	1.6	12.5	36.9	0.88	0.55	0.036
Mid	40.1	20.7	2.0	2.0	12.0	33.9	0.86	1.61	0.142
Historic	38.0	22.2	1.4	0.8	22.0	72.3	—	—	—

Table 3.4 Summary of statistical trends (no trend, increasing, decreasing) of water column TP, TN, and secchi depth according to long-term (> 5 years) monitoring measurements by LAKEWATCH.

Lake	TP	TN	Secchi
Apopka	no trend	no trend	no trend
Cypress	no trend	no trend	decreasing
Doctors	no trend	no trend	no trend
Eustis	decreasing	decreasing	increasing
Glenada	decreasing	no trend	no trend
Griffin	decreasing	no trend	no trend
Istokpoga	increasing	increasing	decreasing
Jesup	decreasing	no trend	decreasing
Kissimmee	no trend	no trend	no trend
Lochloosa	no trend	no trend	increasing
Maitland	n.a.*	n.a.	n.a.
Marian	n.a.	n.a.	n.a.
Orange	no trend	no trend	no trend
Thonotosassa	no trend	no trend	decreasing
Tohopekaliga	no trend	no trend	no trend
Watertown	n.a.	n.a.	n.a.
Weohyakapka	increasing	increasing	decreasing
Yale	increasing	increasing	decreasing

*n.a. indicates no long-term records are available.

Figures

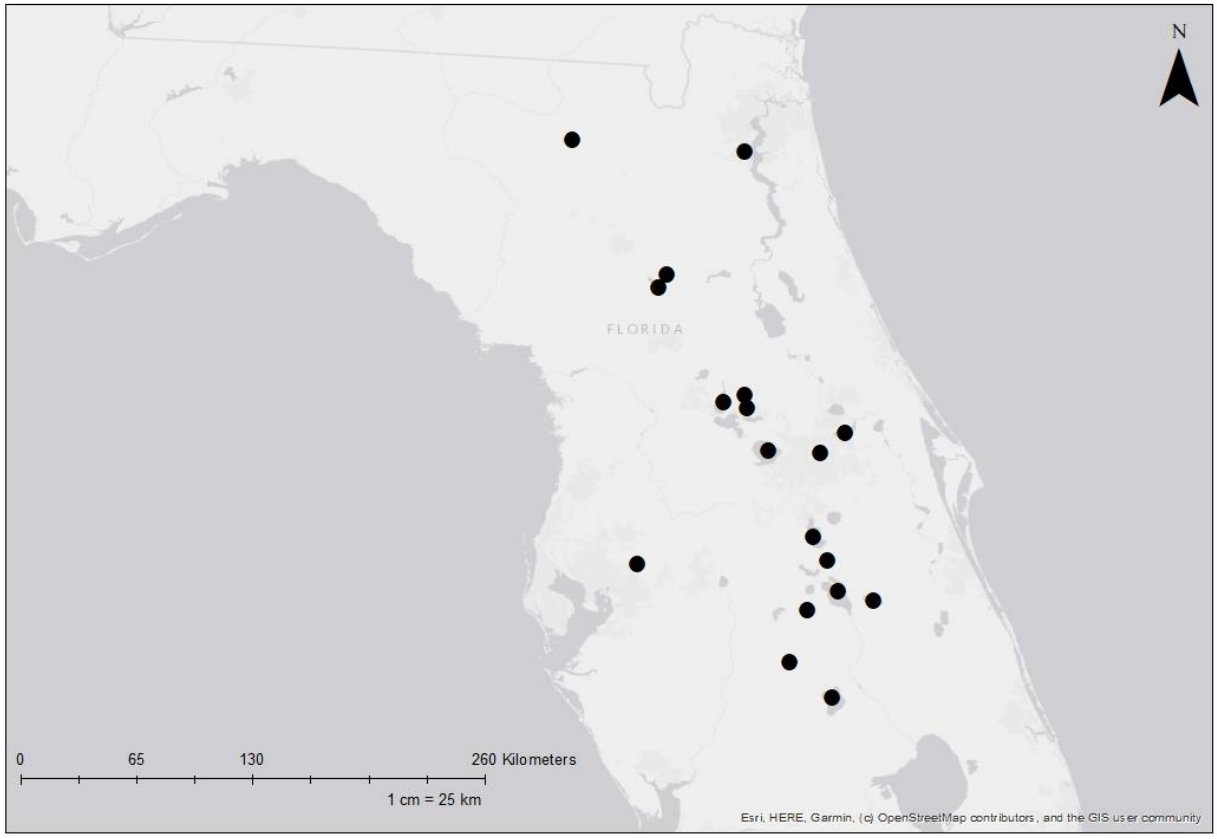

Figure 3.1. Geographic distribution of the 18 study lakes in Florida, USA.

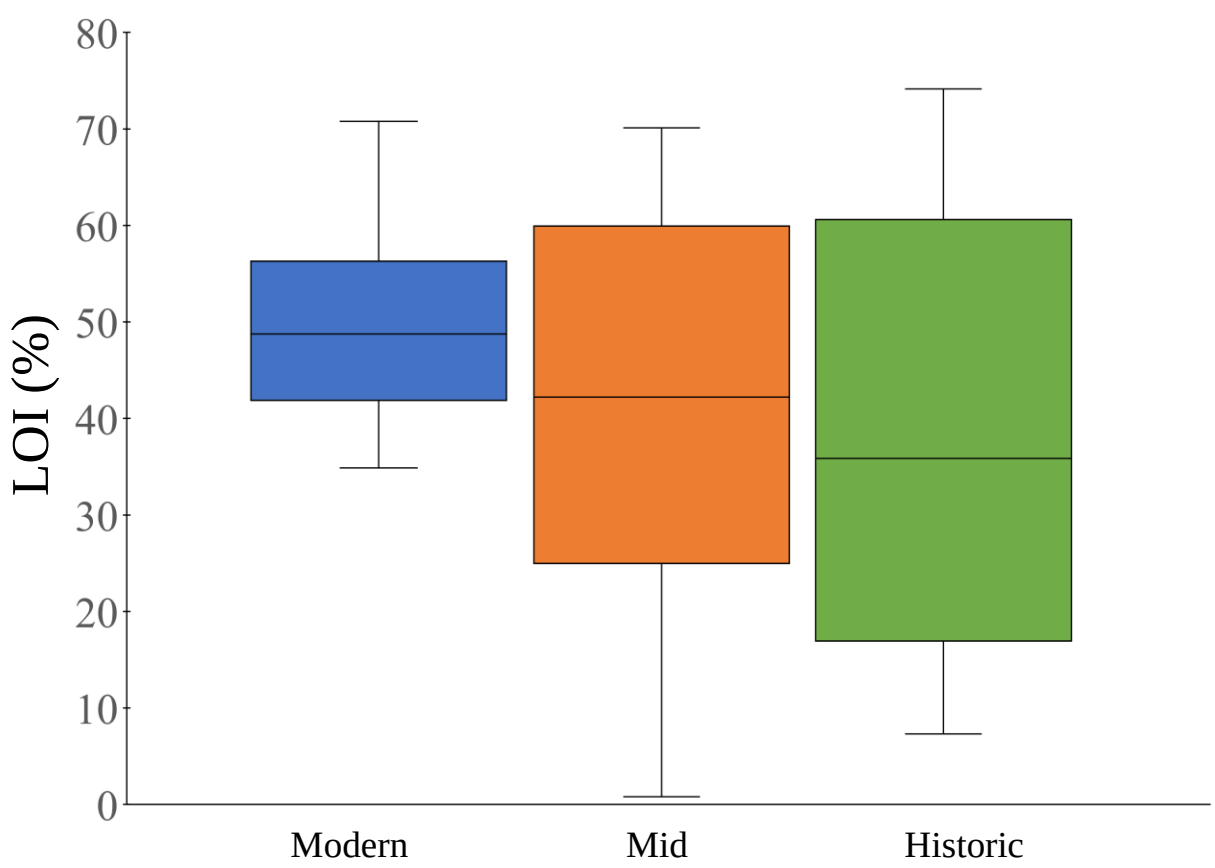

Figure 3.2. Boxplots comparing sedimentary organic content (LOI) as a percent between modern, mid, and historic samples. There are no significant differences between groups (p=0.17).

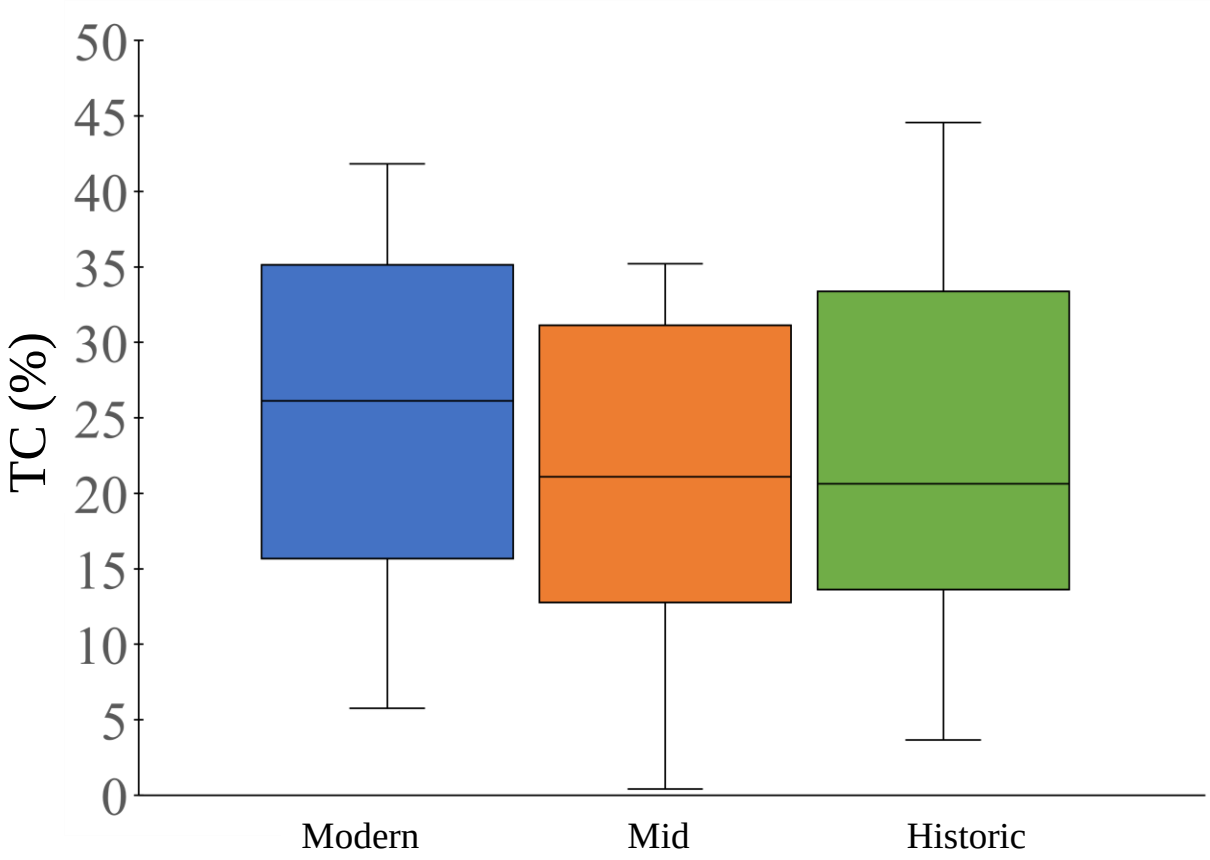

Figure 3.3. Boxplots comparing sedimentary total carbon (TC) as a percent between modern, mid, and historic samples. There are no significant differences between groups (p=0.41).

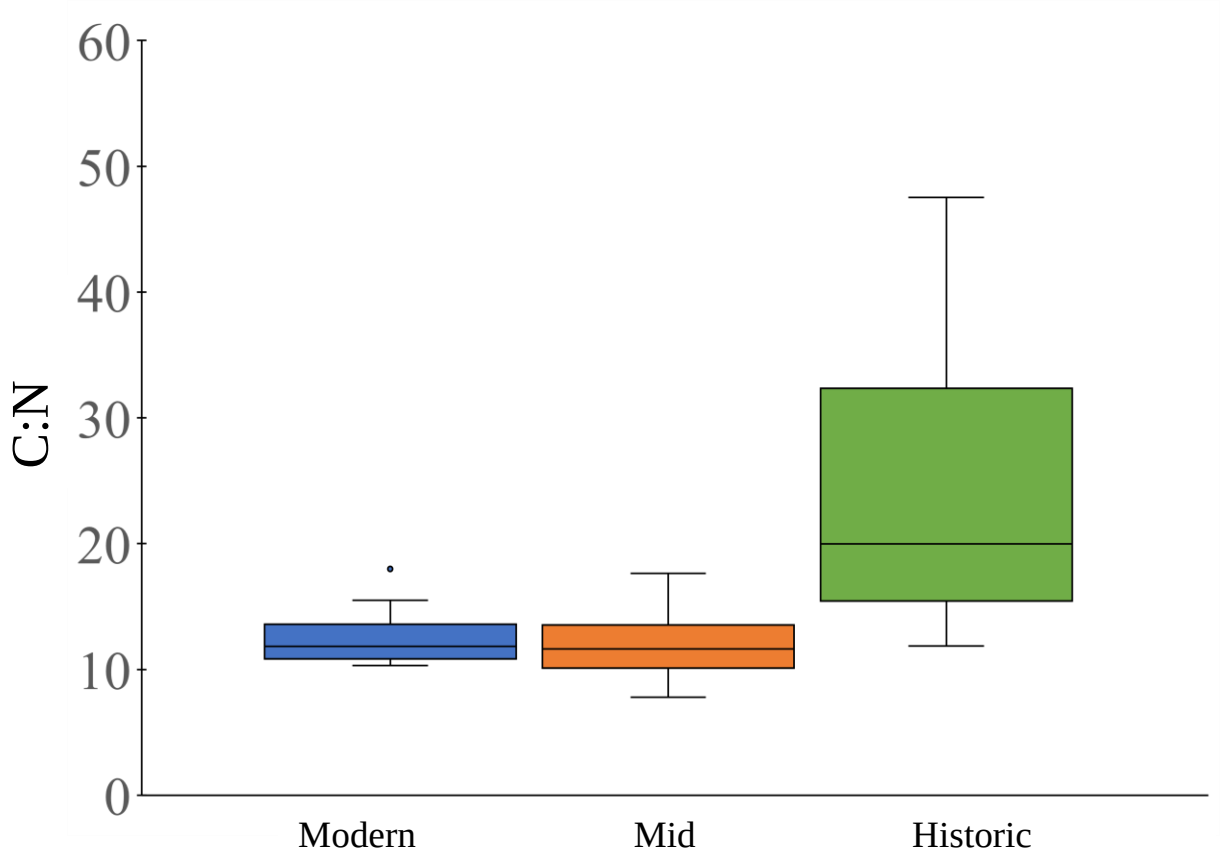

Figure 3.4. Boxplots comparing sedimentary molar stoichiometric ratios of TC and TN (C:N) between modern, mid, and historic samples. Historic samples are statistically significantly higher than modern and mid samples ($p<0.0005$).

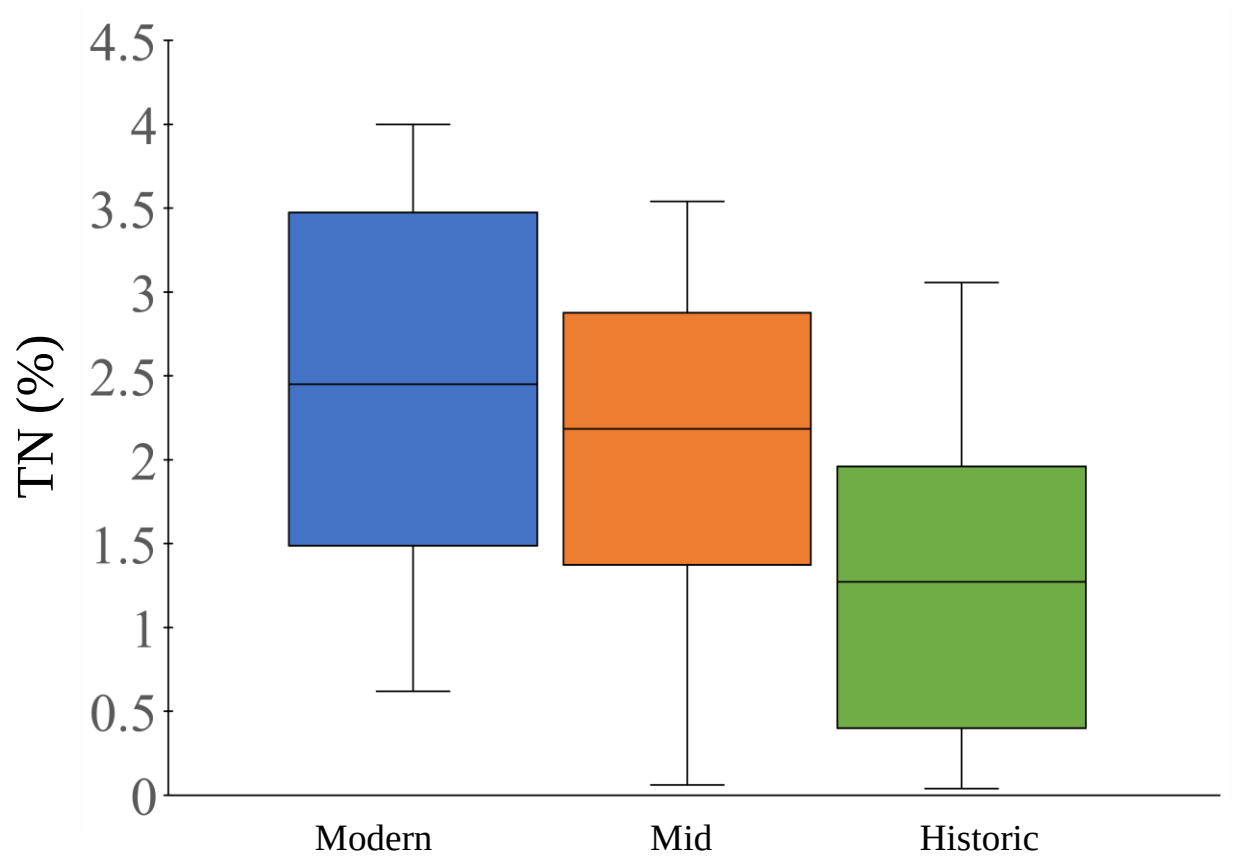

Figure 3.5. Boxplots comparing sedimentary total nitrogen (TN) as a percent between modern, mid, and historic samples. Historic samples are statistically significantly lower than modern and mid samples (p=0.01).

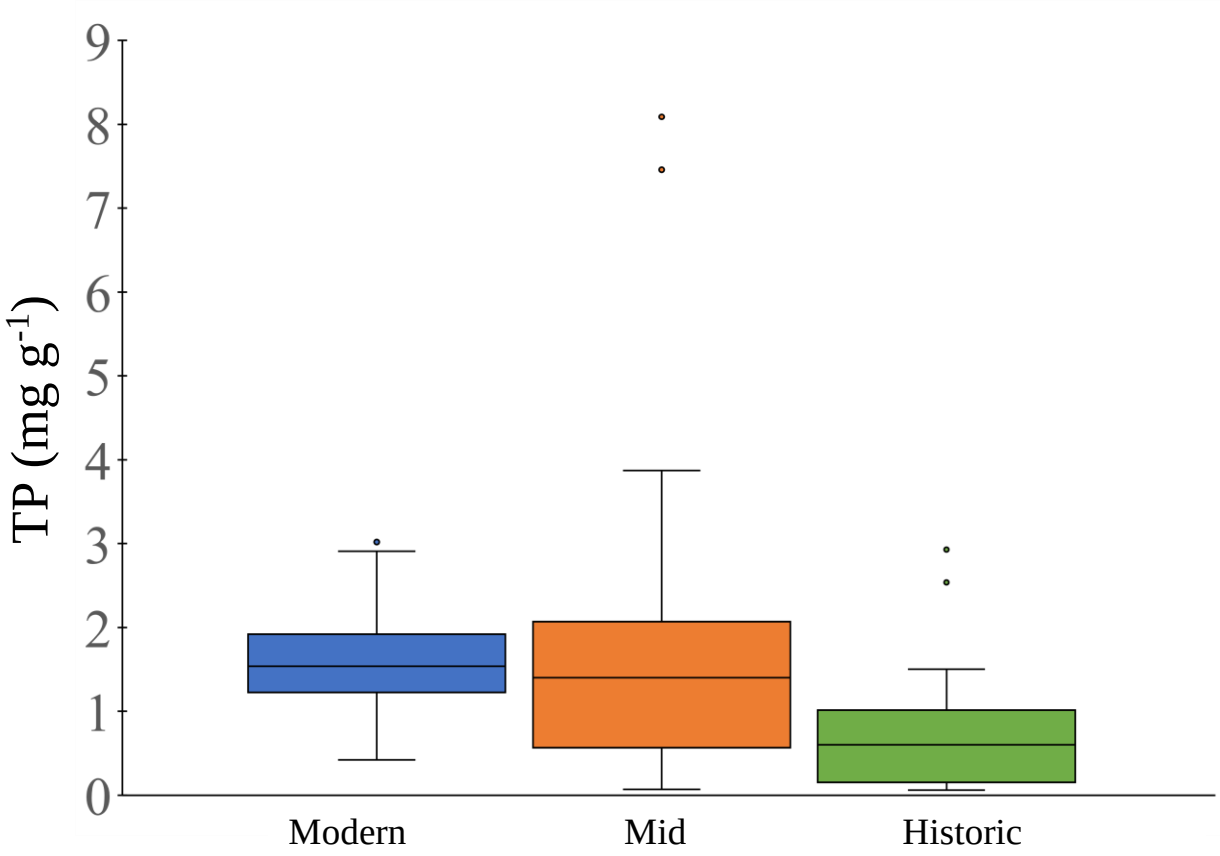

Figure 3.6. Boxplots comparing sedimentary total phosphorus (TP) in mg g^{-1} between modern, mid, and historic samples. Historic samples are statistically significantly lower than modern and mid samples (p=0.04).

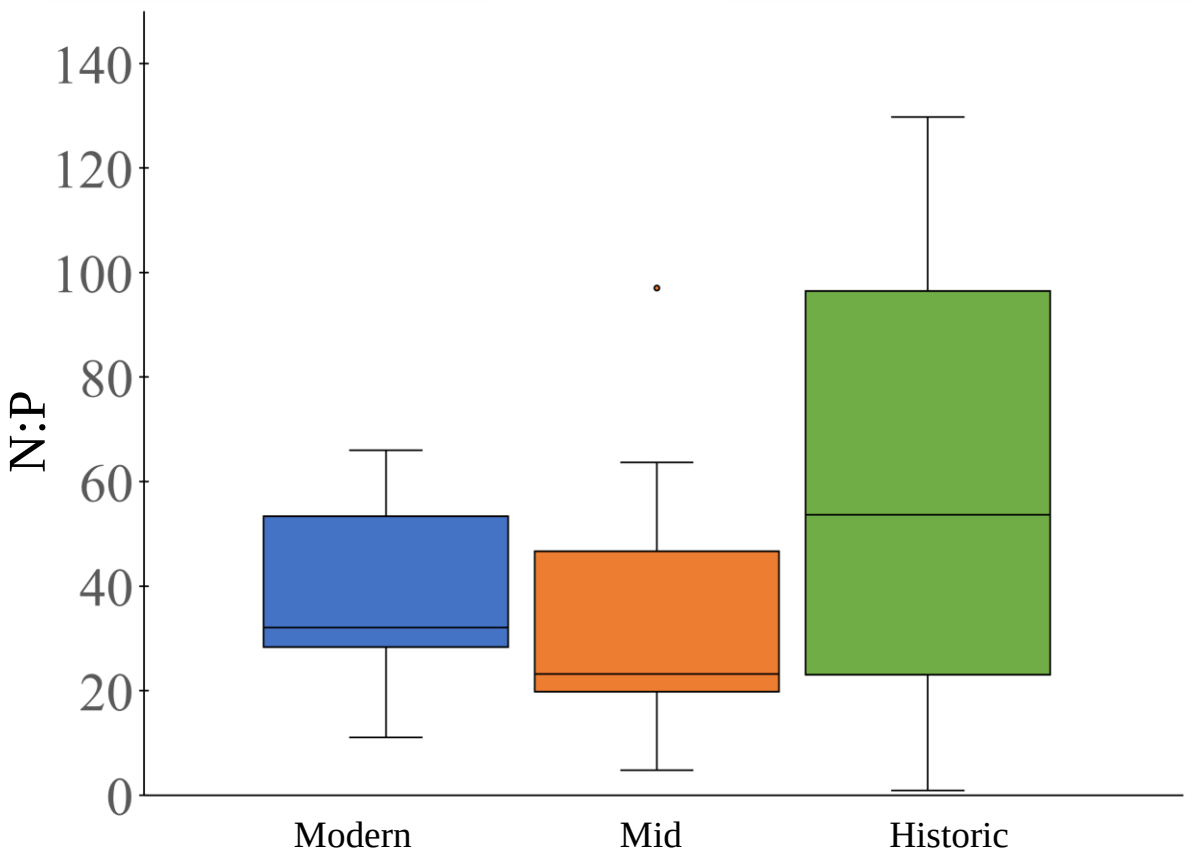

Figure 3.7. Boxplots comparing sedimentary molar stoichiometric ratios of TN and TP (N:P) between modern, mid, and historic samples. Historic samples are statistically significantly higher than modern and mid samples (p=0.01).

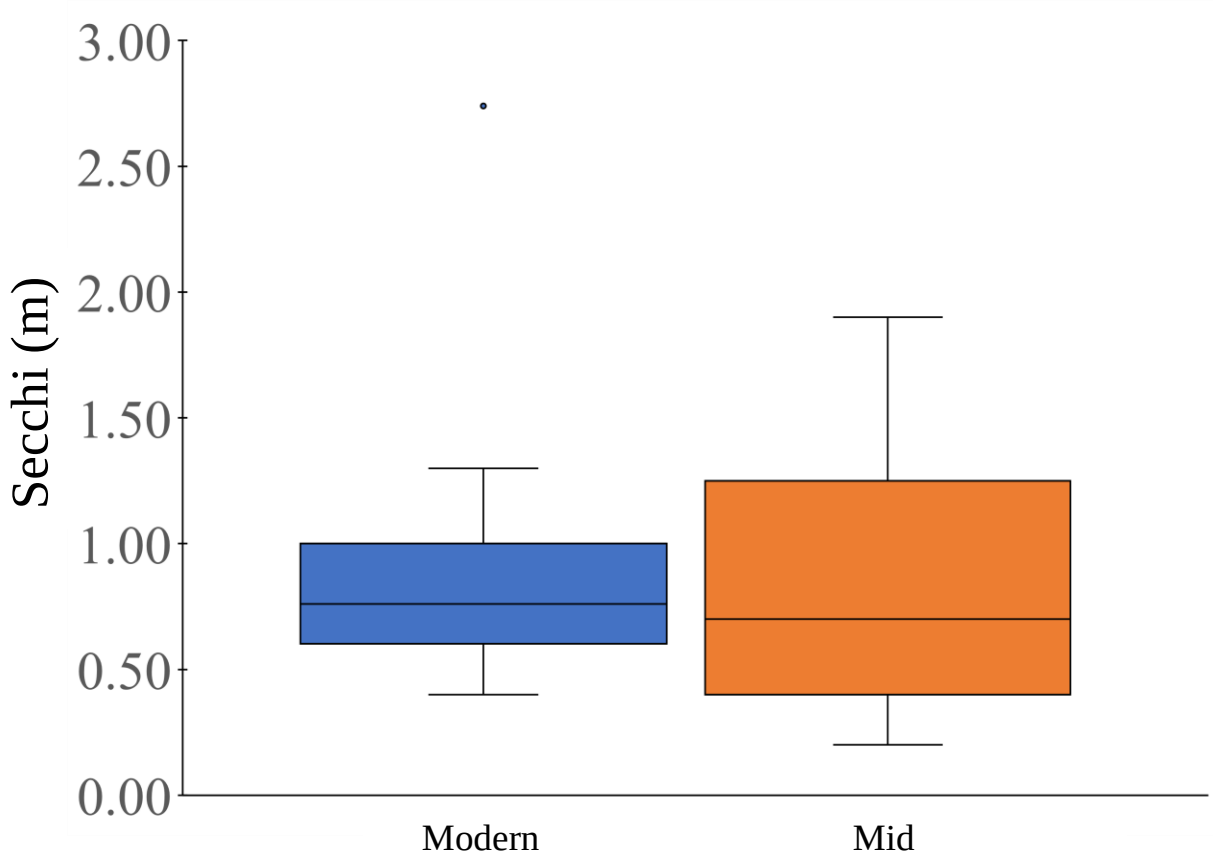

Figure 3.8. Boxplots comparing secchi depth in m between modern and mid water samples. There are no significant differences between modern and mid secchi depths (p=0.45).

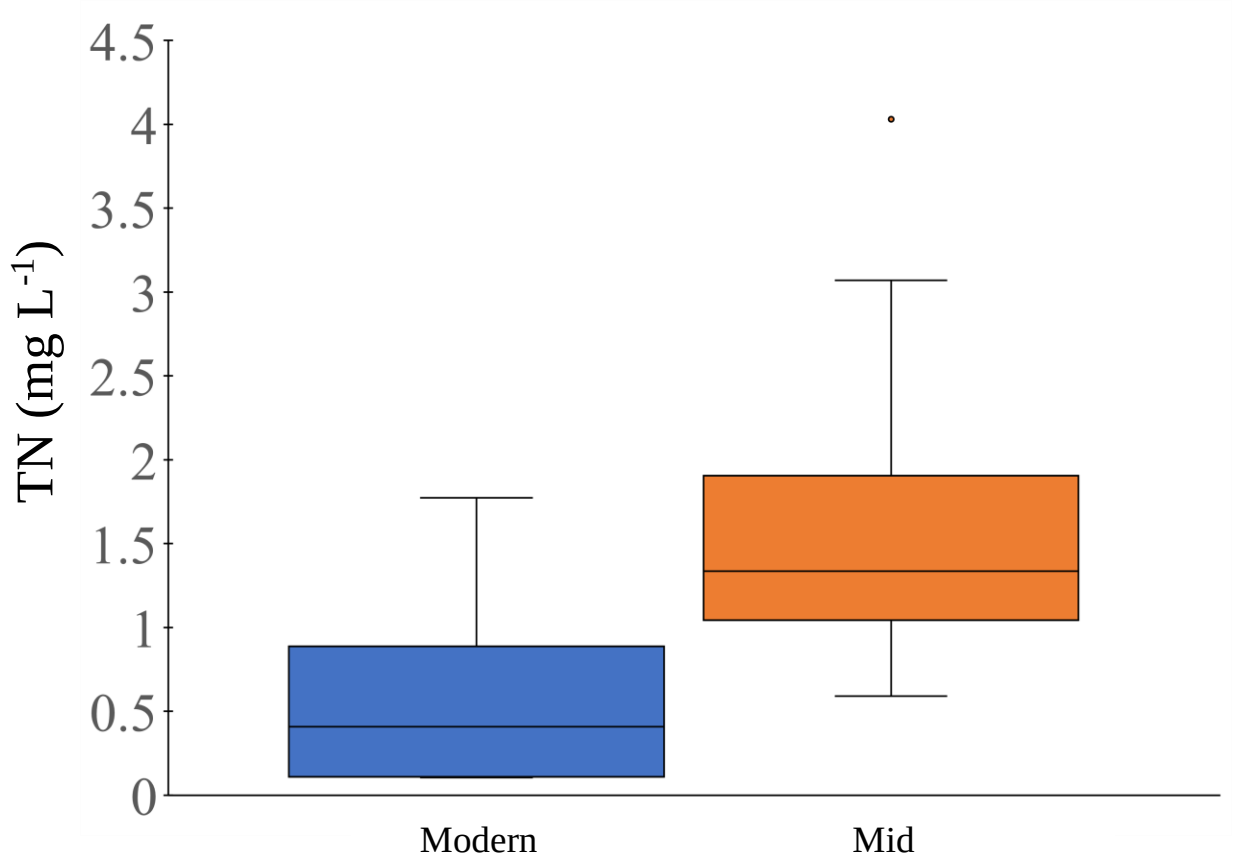

Figure 3.9. Boxplots comparing total nitrogen (TN) in mg L^{-1} between modern and mid water samples. Modern samples are statistically significantly lower in TN concentration than mid samples ($p<0.0005$).

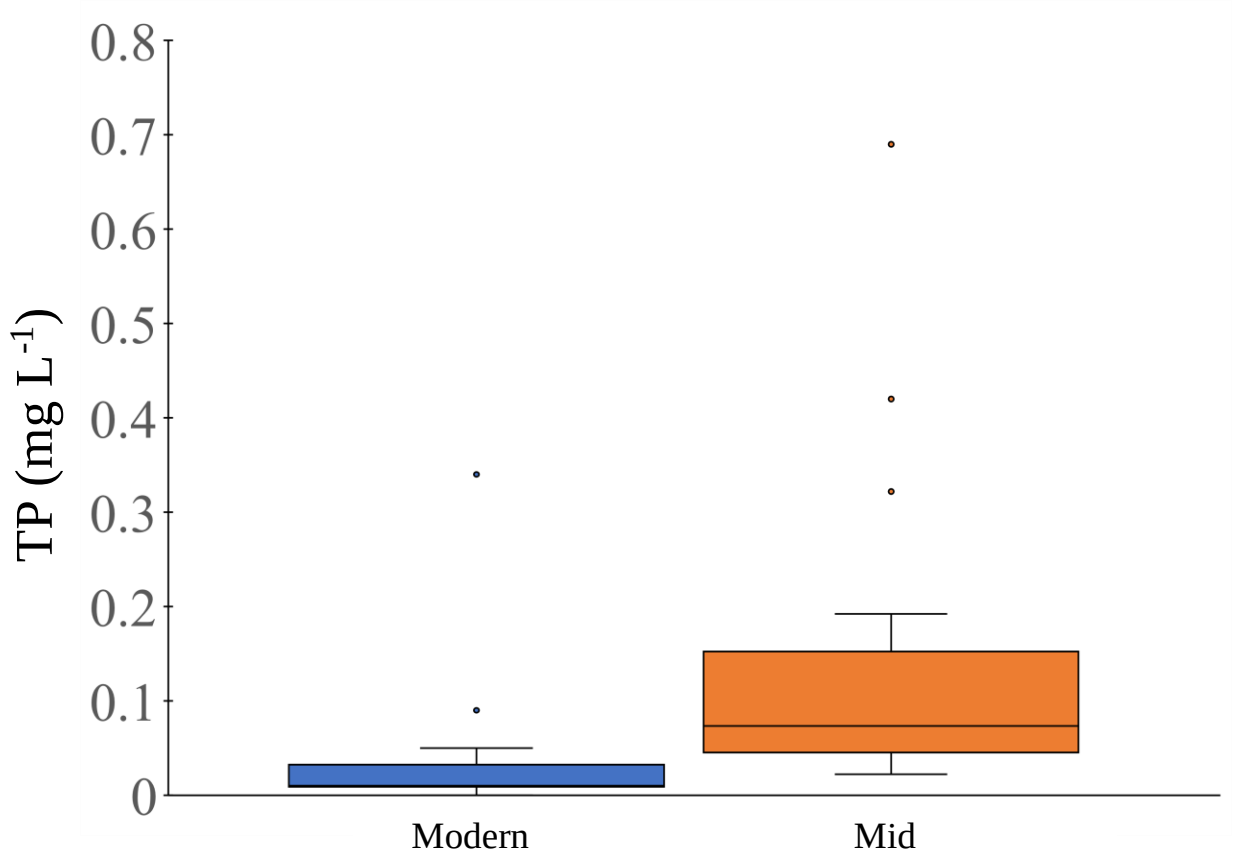

Figure 3.10. Boxplots comparing total phosphorus (TP) in mg L^{-1} between modern and mid water samples. Modern samples are statistically significantly lower in TP concentration than mid samples ($p<0.0005$).