# *Cyanotoxin Production in Subtropical Lakes over the Last 150 Years with Implications for Human and Ecosystem Health*

By

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Abstract

Lakes are experiencing a modern period of increased eutrophication due to cultural impacts and climate change. One common characteristic of lake eutrophication is the increase in cyanobacteria, which possess a competitive advantage over other phytoplankters. This competitive advantage often leads to cyanobacteria dominance and the formation of harmful algal blooms (HABs). HABs can confound ecosystem services through anoxia, biological stress, and the production of toxins, called cyanotoxins, that can be hazardous to ecosystem and human health. Cyanotoxin production within the water column has been widely monitored, however, less work has been done to ascertain the storage and fate of these cyanotoxins within the sediment. Here, I measured a suite of paleolimnological proxies on four sub-tropical lakes in central Florida, USA, to achieve 3 primary research objectives: 1) create a better understanding of cyanotoxin sediment storage over the past ~150 years, 2) determine the probability and ecosystem impacts from cyanotoxin resuspension, and 3) use the sediment record from Lake Carlton as a case study to identify how anthropogenic alterations to connected lakes affected phytoplankton community structure and cyanotoxin production. Sediment cores were collected from Lakes Bonny, Carlton, Marian, and Thonotosassa, and nutrients, photosynthetic pigments, and cyanotoxins were measured throughout each sediment record. Results show microcystins deposited in the greatest concentrations in the sediment over the last ~20 years, but were measurable throughout the sediment record and are not a novel phenomenon of the past few decades. Cyanotoxin storage amounts within each lake suggest that resuspension events from storms are capable of increasing water column toxin values exceeding recommended monitoring thresholds. Finally, Lake Carlton cyanobacteria and toxin dynamics demonstrated the large-scale change shallow lakes can experience in response to multiple human-related stressors. These

results collectively show the importance of understanding sedimentary toxin dynamics in shallow lake systems.

Acknowledgments

First, I would like to thank the National Science Foundation as our source of funding for this project. Secondly, I would like to thank Dr. Matthew Waters for his guidance and mentorship during my time at Auburn University. I would also like to thank my lab mates, the student workers, and our lab technician, Kaye Bowling, for all of their hard work and assistance these past two years. I would not have been able to achieve any of my goals here at Auburn without their help.

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Chapter 1: Cyanobacteria and Cyanotoxins in Water and Sediment

## Cyanobacteria and Cyanotoxins

Cyanobacteria are a diverse group of photosynthetic, prokaryotic organisms that are found in almost every ecosystem on Earth. Cyanobacteria are believed to have developed during the Proterozoic Era around 2.4 billion years ago during the "Great Oxygenation Event" (Hamilton et al., 2016; Garcia-Pichel et al., 2019). Given rapid and simplistic biology when compared to other phytoplankters, cyanobacteria can form dense populations known as harmful algal blooms (HABs). HABs are problematic for lake biota and humans as HABs can reduce light attenuation, create thick surface scums, limit dissolved oxygen concentrations, cause fish kills, and produce cyanobacterial-derived toxins—cyanotoxins (Paerl and Huisman 2009). Microcystin (fig 1.1), the most common cyanotoxin (Sivonen and Jones 1999), is a hepatotoxin known to cause pneumonia-like symptoms, liver failure, and certain cancers (Carmichael 1992). Though most commonly associated with *Microcystis aeruginosa*, other genera, such as Dolichospermum (formerly *Anabaena), Planktothrix*, and *Nostoc,* have also been documented to produce microcystin (Dawson 1998).

In recent decades, cyanobacteria dominance has increased, giving rise to increased HABs (Ewing et al. 2020) and growing concern due to intersections between cyanotoxins and human health risks. Public concern culminated when Lake Erie exhibited high levels of cyanotoxins near a drinking water treatment facility inlet in August 2014, and 500,000 residents of Toledo, Ohio, were issued a "do not drink" warning for their municipal drinking water supply (Toledo, 2014). The United States Environmental Protection Agency estimates that between 30-48 million Americans are drinking water that is periodically contaminated with cyanotoxins (EPA, 2015). Cyanotoxins are difficult and costly to remove from municipal drinking waters with efficiency

rates as low as 60% (Zamyadi 2013). This growing concern has caused an increase in monitoring efforts throughout the USA to identify the presence and concentration of microcystins in natural waters.

Whereas cyanotoxin measurements have increased, cyanotoxin production is poorly understood with multiple drivers suggested in the literature (Henao 2019). Cultural eutrophication seems to play a key role in cyanotoxin production (Dolman 2012), but not all eutrophied systems produce cyanotoxins. Changes in N:P ratios have been shown to stimulate cyanotoxin production (Dolman et al. 2012), as well as negative correlations with nitrogen (Kokocinski 2013). Micronutrients, such as S and Fe, have been shown to increase cyanotoxin production (Utkilen 1995). Alterations in nutrient dynamics are the most cited driver, but biological drivers have also been identified. Zooplankton grazing and allelopathy have also been suggested as biological drivers of cyanotoxin production (Holland and Kinnear 2013).

**Cyanotoxin Measurement in Water and Sediment**

The concern over cyanotoxin occurrence in recreational and drinking source waters has influenced the number of monitoring projects including cyanotoxin measurements as a water quality parameter. Monitoring projects such as the EPA's National Lakes Assessment (Fig. 1.2) and the Florida Department of Environmental Protection (Fig. 1.3, Fig. 1.4) regularly test for cyanotoxins and advocate for using cyanotoxin values to make managerial decisions and relay safety information to the public. However, enumerating cyanotoxin concentrations is time-consuming and expensive, which limits many monitoring programs to minimal sample size and frequency. The National Lakes Assessment, for instance, is a nationwide survey of lakes conducted once every five years. The EPA selected 1000 lakes and each is sampled only once in the summer months. Aquatic ecosystems are highly dynamic with environmental changes

occurring quickly and observable alterations being volatile. As a result, low sampling frequency can fail to record correct and representative samples. Thus, a need for measurement techniques with a broader sampling scale that incorporates a larger temporal and spatial variation is needed. Paleolimnological tools applied to the sediment record can provide historic data that integrates sub-decadal periods per sample, which can trace the history of cyanotoxin production as well as calculate the amount of cyanotoxin stored in the sediment environment.

Lakes act as sentinels and integrators of human impacts on terrestrial and aquatic ecosystems (Williamson 2008). Paleolimnology utilizes lake sediment deposition as a long-term dataset representative of whole-lake conditions, and these sediment records precede monitoring efforts for most lake systems. Sediment deposition integrates sub-decadal periods related to long-term processes instead of daily environmental fluctuations. Cyanobacteria abundance has been inferred using paleolimnological proxies such as photosynthetic pigments (Leavitt and Hodgson 2001), diatom assemblages (Whitmore 1989), and alterations to stable isotope ratios (Brenner et al. 1999). However, few studies have utilized direct measurement of 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], Waters et al. 2021). Matching cyanotoxins, specifically microcystin, with other sediment variables could lead to a better understanding of cyanotoxin fate and transport within the lacustrine system.

Organic carbon burial rates can be calculated using bulk sedimentation rates and organic carbon content in dry sediment (Waters, 2019). Basin-wide accumulation rates are created by establishing sediment focusing factors from expected and observed $^{210}$Pb inventory (Baskaran, 1993). These focusing factors account for soft sediment movement within the lake basin. The

same techniques can be applied to cyanotoxins stored within the sediments. Through the use of focusing factors and bulk sediment accumulation rates, burial rates and basin-wide estimates of cyanotoxin bedload can be established and used to assess the fate and internal transport potential of cyanotoxins.

**Study Sites:**

Due to the karst topography with limestone-rich bedrock, Florida lakes are numerous, shallow, and possess small watershed areas to lake surface area (Wetzel 2001). Due to the large absence of rivers, and the relatively small watersheds of these lakes, Florida lakes are largely fed via rainwater runoff and groundwater (Canfield 1981). Additionally, many Florida lakes sit atop local phosphate deposits that stimulate primary production and are likely to sustain cyanobacterial communities long before anthropogenic influences affected these systems (Carr 1934). Lake morphology also plays a key role in net primary production within these Florida systems. The shallow nature of these lakes makes them susceptible to wind-driven sediment resuspension (Bachmann 2000) allowing for the internal loading of nutrients such as phosphorus from the sediments. This internal nutrient recycling can also contribute to the hypereutrophic nature of these subtropical systems in Florida.

From a National Science Foundation EAGER pilot study of 47 Florida lakes on sedimentary cyanotoxins (Lamb 2021), 4 lakes were selected for this project: Lake Bonny, Lake Carlton, Lake Marian, and Lake Thonotosassa (Fig 1.5). These lakes experience high levels of cultural eutrophication, detectable levels of microcystin within their sediments, and their stratigraphy could be corroborated by previous paleolimnological studies (Brenner et al. 1996, Schelske et al. 2005). They are shallow, hypereutrophic lakes located within Florida's central ridge and overlay the phosphatic deposits of the Miocene Hawthorne Formation (Brooks 1981)

(Figure1.5) (Table 1.2). Lake Bonny is a small, solution depression lake located in Lakeland, Florida, the basin is situated within the highly urbanized Peace River watershed. Lake Marian, in Osceola County, Florida, is situated within the Kissimmee River watershed. The watershed is mostly comprised of agriculture, pastureland, and wetland land cover with one small residential community along the Northeast shoreline. Lake Thonotosassa's watershed is a mixture of urbanized and agricultural land covers in Hillsborough County, Florida. The basin is situated within the Hillsborough Bay watershed. Lake Carlton's immediate watershed is a mixture of residential and urbanized land covers. The basin is part of the Ocklawaha Chain of Lakes and is directly connected via canal to Lake Beauclair. Historically, this chain of lakes has experienced hydrologic changes through an extensive canal network and large-scale agricultural development of floodplain wetlands (Schelske et al. 2005).

**Thesis Rationale and Objective:**

Here, I measured a suite of paleolimnological proxies on four sub-tropical lakes in central Florida, USA, to achieve 3 primary research objectives: 1) create a better understanding of cyanotoxin sediment storage over the past ~150 years, 2) determine the probability and ecosystem impacts from cyanotoxin wind-resuspension, and 3) use the sediment record from Lake Carlton as a case study to identify how anthropogenic alterations to connected lakes collectively affect phytoplankton community structure and cyanotoxin production. Through this investigation, I will reconstruct historic nutrient deposition, primary producer community structure, and cyanotoxin production for each system. These data will allow for a better understanding of how cyanobacteria respond to alterations in nutrient dynamics and primary producer community structures. Tracking these changes along with reconstructing cyanotoxin production could help identify drivers of toxin production and ultimately help identify how these

metabolites are transported and stored in the sediments. The long-term storage of cyanotoxins is poorly understood and understudied. This project will give new insight into whether managers should reconsider how they monitor cyanotoxin abundance and how they could mitigate the health risks associated with them.

**Figures:**

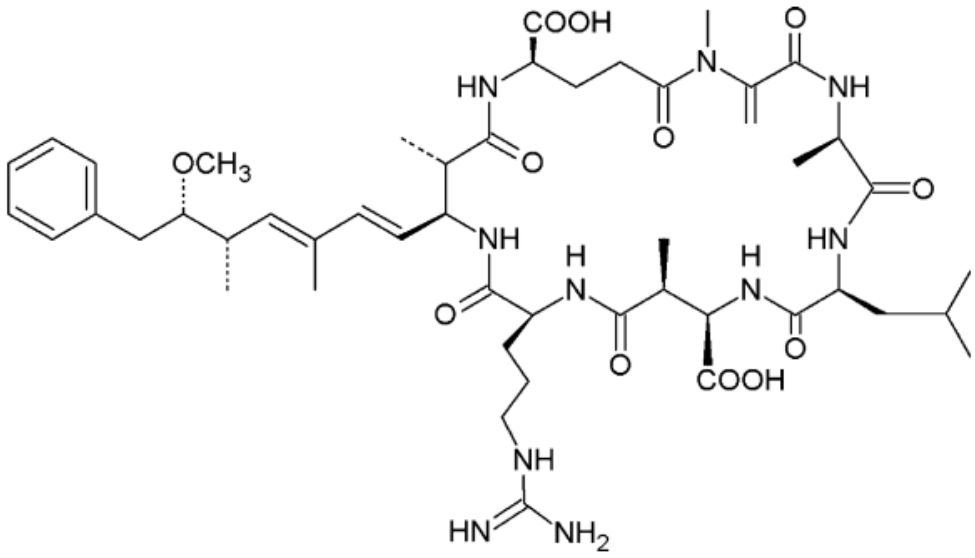


Figure 1.1: Structure of microcystin-LR

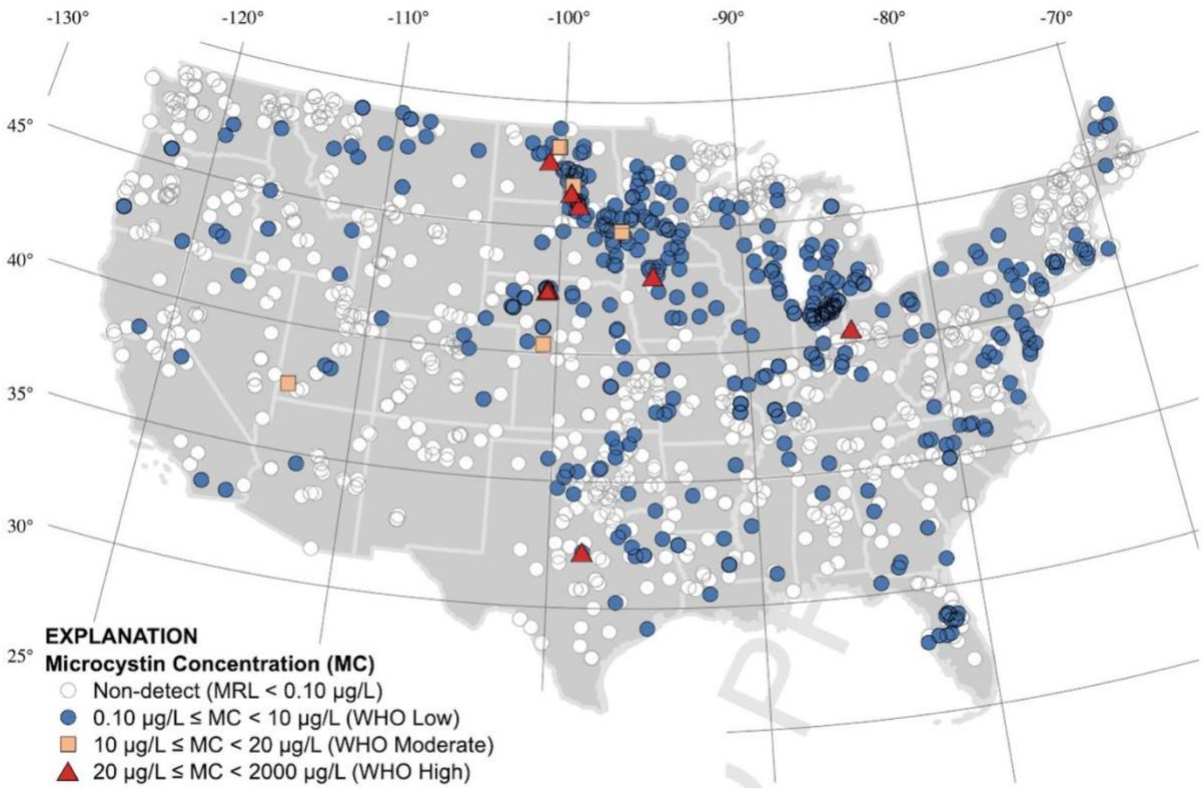


Figure 1.2: This map shows all of the lakes tested by the EPA for the National Lakes Assessment in 2012 and those in which samples contained microcystin (https://abraxis.eurofins-technologies.com/).

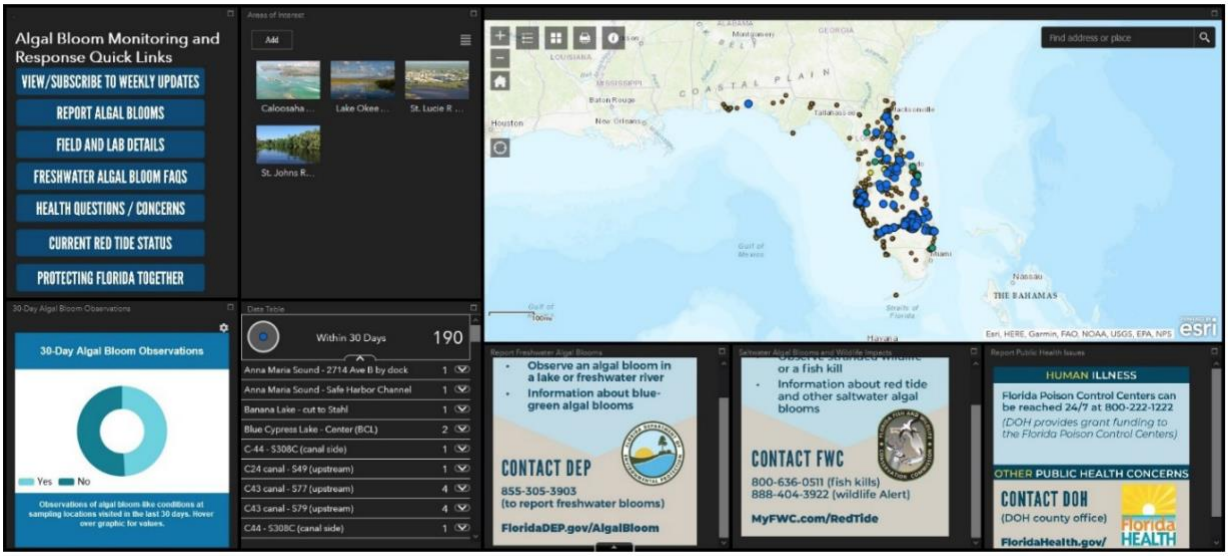


Figure 1.3: the FDEP Algal Bloom Sampling Status Dashboard (www.floridadep.gov/AlgalBloom).

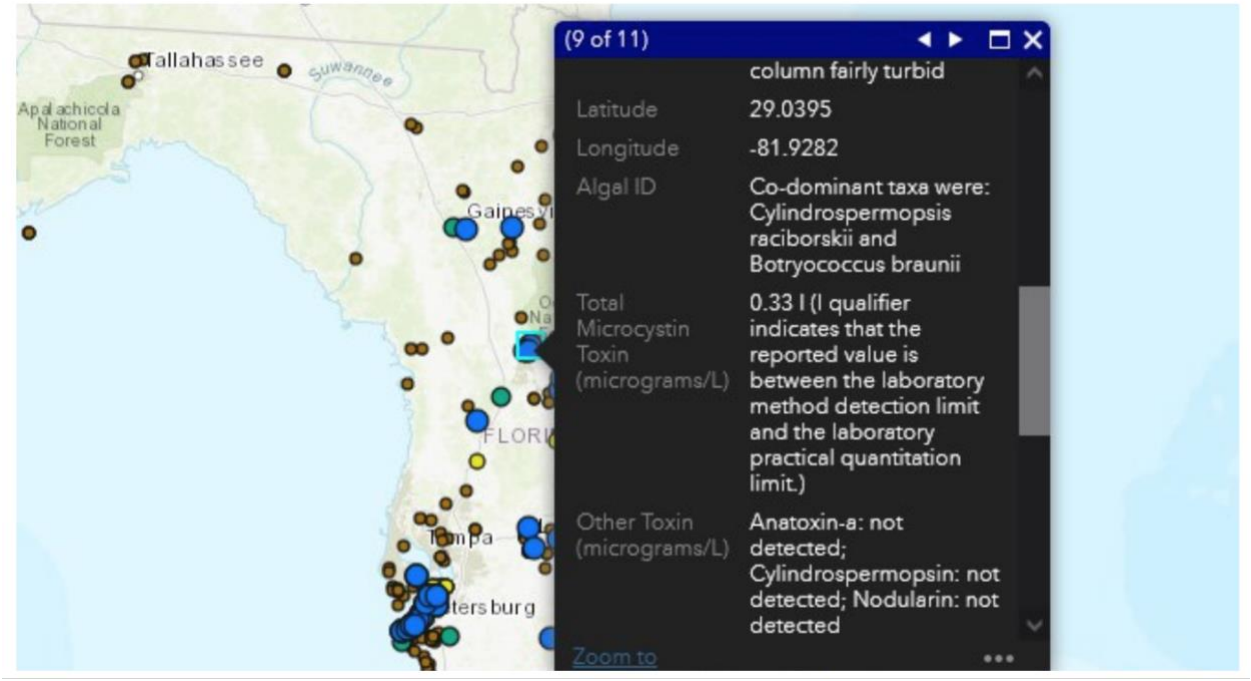


Figure1.4: Screenshot of a report window from the FDEP Algal Bloom Sampling Status Dashboard showing the presence of microcystin found in Lake Weir in Marion County.

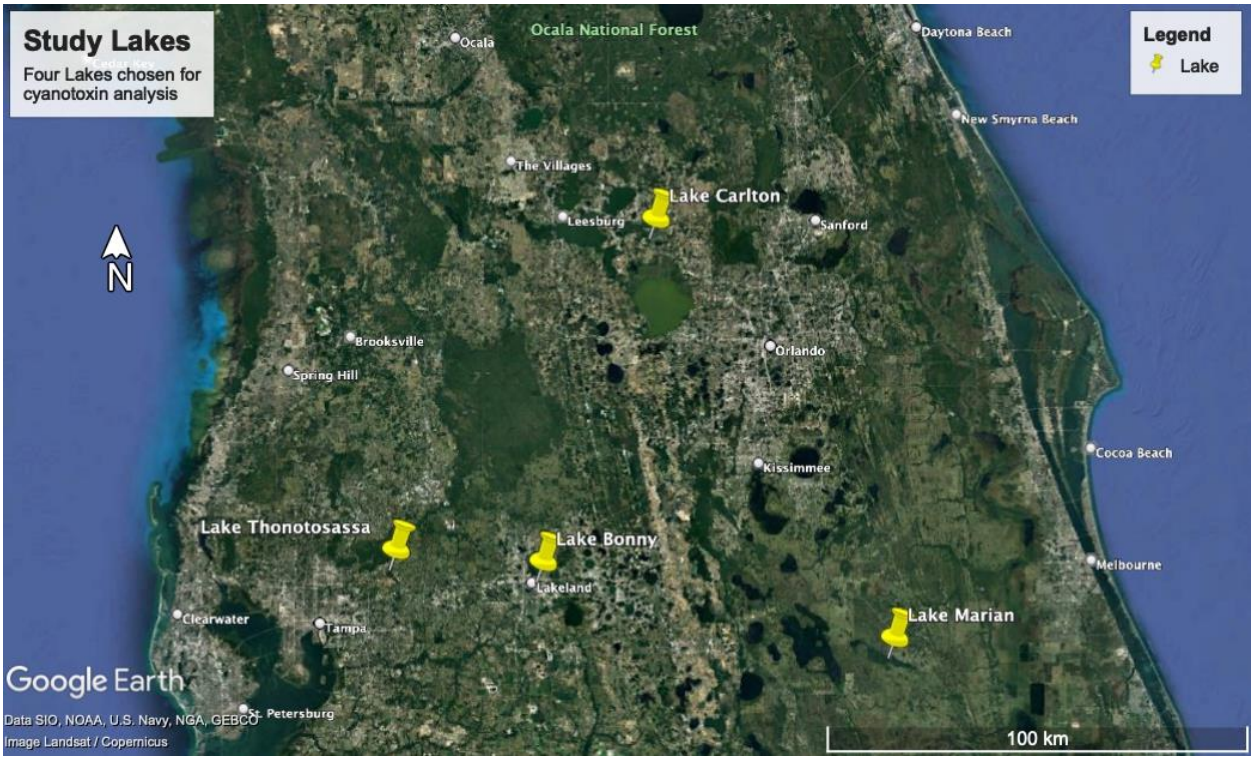


Figure 1.5: Map depicting 4 study site locations along the Central Ridge of Florida: Lake Bonny, Lake Carlton, Lake Marian, and Lake Thonotosassa.

**Tables:**

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

| 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 |
| MC (8 congeners) | Guatemala, Lake Amatitlán | 550 | 2150 BP | LC-MS | 75% MeOH, SPE | Waters et al. 2021 |
| CYN | USA (Florida), Lake Griffin | 300 | 4732 BP | ELISA | 50% MeOH | Waters 2016 |

Table 1.2: Study lake characteristics for each lake cored for this study. Units are surface area (hectares), maxium depth (m), average depth (m), latitude and longitude, land cover description.

| *Lake* | *Surface Area* | $Z_{max}$ | $Z_{mean}$ | *Lat./Long.* | *Land Cover* |
|---|---|---|---|---|---|
| *Bonny* | 82 ha | 3.4 m | <1 m | 28°02'05.3"N 81°55'52.2"W | Urban |
| *Carlton* | 153 ha | 4 m | 4 m | 28°45'36.2"N 81°39'27.5"W | Residential |
| *Marian* | 2323 ha | 5.1 m | 4 m | 27°52'56.6"N 81°06'42.1"W | Agricultural |
| *Thonotosassa* | 332 ha | 4.5 m | 2.5 m | 28°03'N, 82°16'W | Agricultural Residential |

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Chapter 2: Sedimentary Cyanotoxin Dynamics and Potential Internal Loading in Four Shallow Florida Lakes

**Abstract:**

Cultural eutrophication on watersheds and lake ecosystems are characterized by increased nutrient loading and increased dominance of cyanobacteria. This increase in cyanobacteria abundance can confound ecosystem services through anoxia, biological stress, and the production of toxins which can be hazardous to human and ecosystem health. Where human recreation and drinking water sources intersect with cyanobacteria, caution is needed to ensure health risks are minimized for lake users and aquatic biota. While the impacts of cyanotoxins are well known, the environmental triggers of cyanotoxins and the history of cyanotoxins in lake systems are much less understood. To better understand the long-term dynamics associated with cyanotoxin production, I conducted a paleolimnological investigation on four shallow and subtropical lakes in central Florida, USA to accomplish three research objectives: 1) reconstruct the cyanotoxin production history over the last ~150 years, 2) link cyanotoxin deposition with proposed drivers of cyanotoxin production such as cyanobacteria abundance and nutrients, and 3) determine to internal loading of sedimentary cyanotoxins to the water column during resuspension events. To accomplish these objectives, short (~1.5 m) sediment cores were collected from each system and sediments were analyzed for sedimentation rates through excess $^{210}$Pb, cyanotoxins as total microcystins, photosynthetic pigments and nutrients. Results show that sediment deposition of cyanotoxins occurred throughout the last ~150 years for all systems with dramatic increases in the last 30 years. Cyanotoxin production did not coincide directly with cyanobacteria abundance but did follow nutrient patterns of P and had a negative relationship with Fe. Microcystin was stored within the bedload of all four study lakes at levels

well above World Health Organization guidelines for drinking water (<1ug $L^{-1}$) and recreational uses (20 ug $L^{-1}$). Considering the shallow and wind-stressed nature of these lake systems, resuspension calculations showed that most lakes experience annual storm or wind events capable of internally loading sedimentary cyanotoxins to levels that could impact water column measurements.

**Introduction:**

As lakes experience increased eutrophication due to cultural impacts on watersheds and aquatic ecosystems, cyanobacteria are given a competitive advantage over other phytoplankters (Paerl 2008). The increase in cyanobacteria abundance can confound ecosystem services through anoxia, biological stress, and the production of toxins which can be hazardous to human and ecosystem health (Paerl 2008). These cyanotoxins have been shown to cause pneumonia-like symptoms, liver damage, and certain types of cancers as well as impact aquatic biota (Dolman et al. 2012) Where human recreation and drinking water sources intersect with cyanobacteria, caution is needed to ensure health risks are minimized for lake users.

A variety of triggers for cyanotoxin production have been proposed from recent investigations with few suggesting the same environmental conditions. Frequently associated with anthropogenic eutrophication, high concentrations of nitrogen (N) and phosphorus (P), as well as alterations to N/P ratios, have been shown to stimulate toxin production (Dolman et al. 2012). However, not all hypereutrophic systems exhibit cyanotoxins (Holland and Kinnear 2013). Certain micronutrients—S and Fe—have also shown associations with toxin production (Utkilen 1995). Known? nutrient triggers such as zooplankton grazing and allelopathy have also been related to cyanotoxin production (Holland and Kinnear 2013), as well as abiotic factors like temperature and light intensity (Griffiths et al. 2003; Dyble et al. 2006). Whereas individual

experimental evidence appears to have uncovered specific cyanotoxin triggers, a consensus has not been established that allows for predicting cyanotoxin production within whole lake ecosystems.

Many water quality monitoring projects now include water column assessments of microcystins and many laboratory projects are focused on discovering why cyanobacteria produce these metabolites. Few studies have investigated whole-lake ecological drivers, historic production, fate, and transport of cyanotoxins. Lake basins are natural traps for sediments (Smol 2008) with allochthonous and autochthonous materials within the lake eventually settling to the lake bottom where they are stored in the sediments and form a historic record. Paleolimnological techniques exist to reconstruct nutrient inputs (Kenney et al. 2002), algal and cyanobacteria communities (Leavitt and Hodgson 2001, McGowan et al. 2005), and sedimentation rates (Appleby and Oldfield 1983).

Though cyanotoxin measurements in sediments are not common, some paleolimnological studies have incorporated cyanotoxin concentrations as measured proxies. Paleolimnological studies have been conducted on lakes in Poland, the USA, and Canada to track eutrophication and cyanotoxin production temporally (Efting et al. 2011, Kaczorowska and Kornijow 2012, Pawlik-Skowronska et al. 2010, Zastepa et al. 2017[2]). Most studies of cyanotoxin deposition in the sediments have been limited to short (maximum of 50 cm) sediment-water interface cores that span the past ~150 years, but more recent studies have shown cyanotoxin records can span several thousand years (Waters 2016; Waters et al. 2021). For microcystins, a majority of these studies have shown most toxin deposition and storage has occurred within the last 20-30 years.

As cyanotoxins are beginning to be utilized as a sedimentary measurement, the fate of these molecules should also be considered. Wind-driven sediment resuspension has been well-

studied in shallow lakes (Scheffer 2008), and local wind patterns and lake morphology can affect sediment distribution (Whitmore et al. 1996) and nutrient dynamics through resuspension (Qin 2020). In shallow systems, wind-driven sediment resuspension could release microcystin into the water column, yet very few studies have considered cyanotoxin resuspension as a pathway of water column cyanotoxin increases. Systems exhibiting cyanotoxin production and periods of wind-driven sediment resuspension are ideal for examining how these processes could affect water quality and long-term cyanotoxin exposure risks.

Here, I collected sediment cores from four hypereutrophic shallow lakes in Florida, USA and applied the paleolimnological measurements of nutrients, photosynthetic pigments and cyanotoxins. The three research objectives of the study were: 1) reconstruct the cyanotoxin production history over the last ~150 years, 2) link cyanotoxin deposition with proposed drivers of cyanotoxin production such as cyanobacteria abundance and nutrients, and 3) determine to potential addition of sedimentary cyanotoxins to the water column during resuspension events.

**Methods:**

*Study Sites:*

The 4 lakes selected for this project: Lake Bonny, Lake Carlton, Lake Marian, and Lake Thonotosassa were chosen based on results of a National Science Foundation EAGER project investigating surfaced sediments and microcystin concentrations of 47 Florida lakes. These lakes all had high levels of cultural eutrophication, detectable levels of microcystin within their sediments, and their stratigraphy could be corroborated by previous paleolimnological studies. All four lakes are shallow, hypereutrophic and located within Florida's central ridge (Table 2.1). The geologic topography is karst with phosphatic deposits of the Miocene Hawthorne Formation (Brooks 1981). Lake Bonny is a small, solution depression lake located in Lakeland, Florida, and

situated within the Peace River watershed. The watershed is highly urbanized. Lake Carlton is adjacent to Mt. Dora, Florida and is part of the Harris Chain of Lakes including Lake Beauclair, with which it is directly connected. The watershed is a mixture of residential and urbanized land covers. Lake Marian, in Osceola County, Florida, is situated within the Kissimmee River watershed. The watershed is mostly comprised of agricultural, pastureland, and wetland land cover with one small residential community along the Northeast shoreline. Lake Thonotosassa is in Hillsborough County, Florida, and the watershed is a mixture of urbanized and agricultural land covers. The basin is situated within the Hillsborough Bay watershed.

*Sample Collection:*

Sediment cores were collected during the summer of 2020 using a piston-coring device that maintains an undisturbed sediment-water interface (Fisher et al. 1992). Each core was sectioned in the field at 4 cm intervals and stored on ice and in the dark until returned to the lab at Auburn University. Sectioning at 4 cm intervals, as opposed to smaller sections, was based on previous paleolimnological investigations in Florida (Brenner et al. 1999, Schelske et al. 2005, Waters et al. 2005) and allows for sufficient sediment material for the variety of analytical measurements. Sediments from hypereutrophic systems in Florida are subject to wind-driven resuspension (Bachmann 2000). This resuspension can lead to uneven sediment distribution within the basin (Whitmore et al. 1996). Based on information from previous studies, or bathymetric data, areas likely to facilitate sediment accumulation were chosen for coring locations.

*Laboratory Analysis:*

A wet aliquot of known volume was removed from each sample for bulk density and determination of organic matter content. Bulk density was determined by drying the known

volume in a drying oven and reweighing and reported as g dry $cm^{-3}$ wet. Organic matter was determined through loss on ignition (LOI) at 550°C in a muffle furnace for 3 hours and reported as a percent (Hakanson and Jansson 1983). Samples were then frozen, freeze-dried, and ground with a mortar and pestle for further analysis.

Total microcystins were measured using the total microcystin/nodularin ADDA enzyme-linked immunosorbent assay (ELISA) kit commercially available from Eurofins (https://abraxis.eurofins-technologies.com/). Total microcystins were extracted from dried sediment samples by combining the methods provided by Eurofins and from a previous study (Zastepa 2017 [1]). A mixture of 75% methanol and 25% water acidified with trifluoracetic acid, was used as the extraction solution. 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 1.5 mL of water and sonicated to dissolve all contents. The extracted samples were analyzed for microcystins per the instructions of the ELISA kit. Final microcystin concentrations were reported as ng microcystin $g^{-1}$ organic matter.

Fossilized photosynthetic pigments were measured using a high-performance liquid chromatography system (HPLC) following the methods of Leavitt and Hodgson (2001) and Waters (2016). Freeze-dried sediment samples were extracted with a solvent mixture of acetone, methanol, and water (80:15:5), which contains an internal standard (Sudan II: Sigma Chemical Corp.). Extraction was done for 16-24 hours in a -20$^{\circ C}$ freezer. 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 passing through a Phenomenex Luna C18 column and measured using a photodiode array detector coupled with a fluorescence detector. Pigments were identified using retention times and pigment-specific spectra of known standards. Pigments are reported of nmol pigment $g^{-1}$ organic matter.

Samples for radiometric dating were packed in plastic test tubes to a height of ~30mm. Sediment in the tubes was covered with epoxy and stored for >21 days in order for samples to reach $^{226}Ra/^{210}Pb$ equilibrium. An ORTEC Intrinsic Germanium Detector was used to measure $^{210}Pb$, $^{214}Bi$ (i.e. $^{226}Ra$), and $^{137}Cs$ activity by direct gamma counting (Appleby 1986). $^{226}Ra$ activity is used as a proxy for "supported" $^{210}Pb$ activity—the activity generated by *in situ* radium in the sediments. "Unsupported" $^{210}Pb$ activity is calculated as the difference between total $^{210}Pb$ activity and supported $^{210}Pb$ activity. Atmospheric deposition of $^{210}Pb$ occurs at a constant rate and, assuming that the lake morphology remains constant over time, can be used to assign dates to sediment samples through the Constant Rate of Supply Model (CRS) (Appleby and Oldfield 1983). These $^{210}Pb$ inventories were used to determine dates for sediment samples, sedimentation rates, and sediment focusing factors to account for uneven sediment distributions within lake sediments (Binford and Brenner 1986).

Carbon and nitrogen were analyzed using a Costech Combustion Elemental Analyzer with an attached autosampler. For organic carbon, samples were acidified in HCl vapor for 24 hours prior to analysis. Phosphorus, iron, and additional elements were analyzed using ICP-ARL following acid digestion in a heated block following EPA method 6010B.

Assessments of wind-driven resuspension were made for each lake using local wind data from the National Climate Data Center (NCDC) and lake morphological data from the Florida Department of Environmental Protection. Following the methods of Bachmann et al. (2000), wave base depth and wind-driven disturbance potentials were determined with the following equation:

$$L_w = 1.56[0.77\ W \tanh[0.077(\frac{9.8F}{W^2})^{0.25}]]^2$$

Where $L_w$ is the wavelength, W is the wind velocity (m/s), and F is the fetch (m). Wave base was described as one-half the wavelength. The dynamic ratio was also determined for each lake, where the square root of surface area ($km^2$) is divided by the mean depth (m).

$$R_d = \frac{\sqrt{S_A}}{Z_{mean}}$$

Due to the low bulk density and flocculent nature of sediments, cyanotoxin concentrations from the top 20 cm of each core were averaged and used to describe toxin bedload and assess potential water column toxin concentrations due to bedload resuspension (Table 2.3).

**Results:**

Lakes Bonny, Carlton, and Marian were dated using the Constant Rate of Supply Model outlined in Appleby and Oldfield 1983. Excess- $^{210}Pb$ dates provide a high-resolution age-depth relation for recent sediment from three of the four study lakes (Fig 2.1). Excess- $^{210}Pb$ activity reached supported levels at 104 cm, 69 cm, and 152 cm for Lakes Bonny, Carlton, and Marian respectively. Lake Thonotosassa could not be dated due to strong groundwater influences

distorting the $^{210}$Pb profile. Instead, LOI data from a previous study (Brenner et al. 1996) was matched to my core to establish a timeline for pre- and post-human disturbances to the watershed. This differentiation occurred at 100 cm in my core and represents ~1900 (Brenner 1996). All of the study lakes are hypereutrophic with high sedimentation rates. Due to these high sedimentation rates and the high level of wind disturbance, sediment focusing factors were developed using the methods of Binford and Brenner (1986). These focusing factors account for irregularities in sediment accumulation and allow for a more accurate representation of whole-lake sedimentary nutrient and cyanotoxin loads.

While the highest microcystin concentrations were observed within the first 30 cm of the cores, measurable amounts of microcystins were found at different intervals throughout the sediment records for each of the study lakes (Figure 2.2; Figure 2.3). The highest cyanotoxin concentrations were recorded in Lake Bonny at 4628 ng $g^{-1}$ org. Lakes Thonotosassa and Marian also exhibited concentrations >4000 ng $g^{-1}$ org , while Lake Carlton had a smaller peak at 754 ng $g^{-1}$ org. Lakes Bonny and Carlton exhibited a series of peaks in microcystin concentration, which become more evident on a log scale (Fig 2.3). Increased microcystin deposition began ~1970 for all the study lakes, but peak microcystin deposition has occurred in the last 2 decades for Lakes Carlton. Marian, and Thonotosassa, with Lake Bonny exhibiting a peak ~1990.

All four of the study lakes exhibit high levels of nutrient deposition throughout each core and are considered hypereutrophic. Lakes Carlton and Marian show continual P loading throughout the cores with maximum values reaching 2.4 and 3.1 mg $g^{-1}$. Bonny and Thonotosassa show large peaks further down the cores (~1950 for Lake Bonny and shortly after 1900 in Lake Thonotosassa) followed by consistent, elevated concentrations reaching 5.1 and 10.6 mg $g^{-1}$ in the top samples, respectively. C and N concentrations are constant until the turn

of the century for all study lakes, where both C and N exhibit sharp decreases in concentration followed by steady increases in concentration to present day.

After 1900, all four study lakes experienced sharp increases in total fluorescence, an indicator of total primary producer abundance. This increase in abundance corresponded to the increased nutrient loading that also occurred during this time. This net increase in total primary productivity is reflected in individual pigment records as well. Diatoxanthin (diatoms) generally spikes around ~1900 in our study lakes and continues to increase to a peak of ~1970. After ~1970, diatoxanthin decreases and is replaced by increases in cyanobacterial pigments such as aphanizophyll and echinenone. Alloxanthin (cryptophytes) exhibits the same spike ~1900 and then generally increases through time, but has an order of magnitude increase in the uppermost section of Lake Bonny. Echinenone (associated with Microcystis cyanobacteria and possibly microcystin production) exhibits smaller peaks ~ 1900 and before 1970, but reaches its maximum after 1970 in the study systems. These trends are matched by aphanizophyll (N-fixing cyanobacteria). Canthaxanthin (cyanobacteria) shows peaks at ~1900 and ~1970 but is then outcompeted by cyanobacteria producing aphanizophyll and echinenone in the upper sediments.

Dynamic ratios for the four study lakes suggest strong wind-disturbance influences are possible for each lake (Table 2.1). Lake-specific wave bases for average and extreme conditions were calculated using local wind speed and prominent wind direction data from the NCDC. These calculations showed that average daily wind conditions (a function of average speed and average direction) cannot generate wave bases deep enough to reach the mean depth of any of the study lakes. These daily summary wind speeds highlight windy days and storm events. This shows that on an average day wind-driven resuspension cannot disturb sediments at or below the mean depth of the lake, though there could be sediment resuspension around the perimeter of the

lake or in shallow areas. However, storm events and other wind episodic events were considered by looking at maximum daily averages. When considering these events, Lakes Bonny and Thonotosassa were suspended 9 days each year while Carlton and Marian were suspended 16 days per year (Table 2.3). To determine the impact these wind events had on cyanotoxin values, cyanotoxin storage was calculated for each lake by using averaged cyanotoxin concentration values and sedimentation rates for the top 20 cm of each core. As a result, during the wind events described above, the sediment internal inputs are capable of increasing water column cyanotoxin concentrations by 80 ug $L^{-1}$for Lake Thonotosassa, 40 ug $L^{-1}$ for Lake Marian, 1.84 ug $L^{-1}$ in Lake Carlton, and 0.84 ug $L^{-1}$ in Lake Bonny (Table 2.3).

**Discussion:**

Each of the study lakes exhibited high concentrations of sedimentary cyanotoxins near the top of each core representing the last 2 to 3 decades, as well as stratigraphic variation throughout each lake's history. Though no one variable can explain increases in cyanotoxin production, nutrient dynamics and cyanobacterial community assemblages demonstrate similar stratigraphies to cyanotoxin deposition. Cyanotoxins are stored in the sediments of these hypereutrophic systems in Florida and are capable of internally loading during intense wind events. The sedimentary flux of cyanotoxins must be considered by lake managers as shallow lake systems are susceptible to wind-driven sediment resuspension (Bachman et al. 2000). Wind-driven resuspension of cyanotoxins can be quantified and compared to local wind patterns to determine risks associated with sedimentary cyanotoxin storage.

*Cyanotoxin Histories*

Each of the study lakes exhibited episodic periods of cyanotoxin production. Spikes occurred throughout the cores, however, peak concentrations occurred within the first ~30 cm.

These peaks corresponded to different time periods for each lake showing Lakes Bonny, Carlton, Marian, and Thonotosassa experienced a heightened period of cyanotoxin deposition beginning in 1996, 2005, 2007, and 2009 respectively. Diagenetic processes could cause sharp decreases in cyanotoxin concentrations in deeper sediments, however, microcystins are chemically stable. Their toxicity remains after boiling (Harada, 1996) and can persist for years when stored dry and at room temperature (Metcalf et al. 2012). Rapid chemical hydrolysis is also unlikely outside of laboratory conditions (Harada, 1996). However, bacteria capable of decomposing microcystin have been documented in sewage effluent, lake water, and lake sediment (Holst, 2003; Edwards, 2008). Other paleolimnologic proxies, such as fossil pigments, are more labile but are still well recognized to produce accurate assessments of lake history. Previous studies have also produced cyanotoxin records spanning thousands of years (Waters 2016; Waters et al. 2021).

Previous work on sedimentary cyanotoxins demonstrates similar stratigraphic changes within the $^{210}$Pb record. Waters (2016) and Waters et al. (2021) both exhibit high concentrations of cyanotoxins near the top of their cores. These higher concentrations are followed by periods of low toxin concentrations with cyanotoxins increasing in deeper sections of the cores outside of the $^{210}$Pb record. All four cores in my study have periods of high toxin concentrations within the top ~30 cm. These highest concentrations are followed by continual fluctuations in cyanotoxin concentration but do not reach the magnitude of concentrations reached in the upper sections of the cores (Fig 2.2; Fig 2.3). Likewise, other records covering similar time periods that measure microcystins have demonstrated similar trends with dramatic increases in the last few decades (Efting et al. 2011, Zapesta et al. 2017). When considering longer timescales, previous studies have also produced cyanotoxin records spanning thousands of years (Waters 2016; Waters et al. 2021). While the increased detection of microcystins has occurred through monitoring efforts,

these sediment data from my study demonstrate that sediments are a viable analytical tool to detect microcystin production. Monitoring efforts generally sample using monthly or even annual timeframe, which can lead to sporadic detection. However, the sediments provide an integrated sampling capable of showing microcystin production throughout a longer time period and could be used to better understand microcystin occurrences in lake ecosystems.

*Cyanotoxins and other paleolimnological proxies*

While sedimentary cyanotoxin interpretations are still being developed, my measurement of total microcystins suggests that cyanotoxin production began with the onset of P inputs from hydrologic changes such as canal construction but reached higher levels in only the last few decades. Through ordination analysis, relationships can be seen and interpreted to determine what variables have direct effects on cyanotoxin production (Fig 2.16). The primary nutrients that ordinated with cyanotoxin concentration were P and Fe. P exhibited a positive relationship, while Fe showed a negative relationship. Both of these trends have been noted in other paleolimnological cyanotoxin studies (Waters 2016, Avery 2020). Micronutrient availability has been shown to influence cyanotoxin production previously, however, these results are conflicted. 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. Echinenone is a photosynthetic pigment thought to be associated with cyanobacteria capable of producing microcystin (Leavitt and Hodgson 2001, Waters et al. 2021). My results showed a positive relationship between microcystin and echinenone, but peak microcystin concentrations did not correspond with peak concentrations of echinenone (Fig 2.12-Fig. 2.15).

*Cyanotoxin resuspension and internal loading*

Sediment studies from other Florida lakes have demonstrated accurate paleolimnological histories despite resuspension events linked to shallow lake depths (Whitmore et al. 1996, Kenney et al. 2002, Schelske et al. 2005, Waters et al. 2015). Previous work by Whitmore et al. (1996) clearly demonstrates that sediments can accrue on the deepest, windward side of these shallow, wind-stressed systems. This sediment focusing can lead to bias in concentration estimates as sediment can either be moving away from or towards the coring location. In order to account for this, a sediment focusing factor must be created and applied to any storage calculations for them to be accurate. This can be achieved by applying a sediment focusing factor by multiplying the sediment accumulation rate with the ratio of the expected, regional unsupported $^{210}$Pb (33.5 dpm cm$^{-2}$) inventory to the observed $^{210}$Pb inventory (Hobbs et al. 2013). This method is commonly used to assess carbon sequestration in lake systems (Heathcote et al. 2015; Waters et al. 2019).

While sediment records can be used to provide whole lake storage of elements such as C (Waters et al. 2019), very few studies have considered whole lake sedimentary impacts of cyanotoxins. Combining cyanotoxin values with resuspension events could provide internally loaded sources of water column cyanotoxin concentrations for monitoring efforts on shallow lakes. For example, multiple monitoring programs currently include cyanotoxin measurements (EPA National Lakes Assessment, Florida LAKEWATCH) with measurements showing sporadic occurrences of cyanotoxins thus causing difficulty in determining related limnological factors. The cyanotoxin storage measurements calculated here showed amounts capable of impacting water column measurements upon resuspension.

Key factors such as sedimentary cyanotoxin concentration, depth, and wind events influence internal loading. The four lakes studied here stored high amounts of cyanotoxins in

their sediments ranging from 0.83 kg to 2363 kg of microcystin per lake (Table 2.3). Some lakes are at a higher risk of resuspension due to local wind patterns and lake morphology (Bachman et al. 2000), but each of the study lakes was subject to frequent, wind-driven resuspension. Though the frequency and intensity of resuspension varied, each system could achieve full lake resuspension 9-16 days a year (Table 2.3). Full lake resuspension has the ability to increase water column concentrations to levels above World Health Organization (WHO) recreational limits of 20 ug $L^{-1}$. Lakes Thonotosassa and Marian frequency release >80 ug $L^{-1}$ and >40 ug $L^{-1}$ respectively due to wind-driven resuspension (Table 2.3). Even in systems where resuspension cannot exceed WHO recreational limits, increased exposure to cyanotoxins could cause bioaccumulation issues in gamefish and other lake biota. Each lake in this study was shallow ($Z_{mean}$ < 4m), but there was large variation in fetch, surface area, and orientation. The smallest of these lakes, Lake Bonny (0.82 $km^2$), has a crescent shape with the longest fetch-oriented NW to SE. The largest lake, Lake Marian (23 $km^2$), has an elliptical shape giving it a long fetch-oriented NW to SE as well. The elongated shape of these lakes gives them large fetches relative to their size. This is the opposite of the other two lakes, Lakes Carlton (1.53 $km^2$) and Thonotosassa (3.32 $km^2$). The circular shape of these two lakes gives them shorter potential fetches as there are no elongated portions of the lakes skewing the distance wind can travel over the surface of the lake. These subtle differences in lake morphology are important to consider when determining resuspension potentials (Bachmann et al. 2000). Long, narrow systems like Lake Marian can have large fetches, however, depending on the average wind direction, these large fetches could be negated through wind crossing perpendicular to the largest fetch. However, even if wave bases cannot extend into the deeper portions of the lake, most organisms will inhabit the shallower, littoral portions of the lake as there is more diverse habitat available. These shallow

lake edges are susceptible to resuspension and could lead to greater bioaccumulation in lake biota.

These sub-tropical systems are also in a region frequently affected by severe weather, tropical storms, and hurricanes. My results show that observed daily wind speed averages have the potential to resuspend sediment in all four study lakes (Table 2.2). With hurricane intensity predicted to increase (Trenberth 2018), this problem could be exacerbated by current anthropogenic alterations to climate.

Given concerns over toxicity and exposure risks associated with microcystins and other cyanotoxins, wind-driven resuspension should be considered when designing monitoring programs and recreational advisories for cyanotoxins. Low sampling frequencies can lead to sampling bias as investigators are unlikely to target windier days. This calm day sampling bias could give an unrealistic understanding of average water column cyanotoxin concentrations. As sediment resuspension releases particulate matter from the lake bottom, this material is likely to settle before monitoring sampling occurs. To address these issues, increased sampling frequency during wind events and increased sediment sampling could provide additional information for managers to make more informed decisions.

**Conclusion**

Though the exact trigger for cyanotoxin production is still unknown, these metabolites pose serious health risks to lake users and lake biota. All of my study lakes have the potential to release microcystin from their sediments multiple days per year given local and observed wind patterns. Two of these lakes have the potential to release cyanotoxin concentrations that exceed the World Health Organization's recreational limits. As many of the lakes in Florida are subject to wind-driven sediment resuspension, sedimentary cyanotoxin loads should be taken into

account when drafting health advisories and recreational guidelines. Random water sampling may not be enough to accurately assess the risks associated with a given water body depending on its cyanotoxin bedload.

**Figures:**

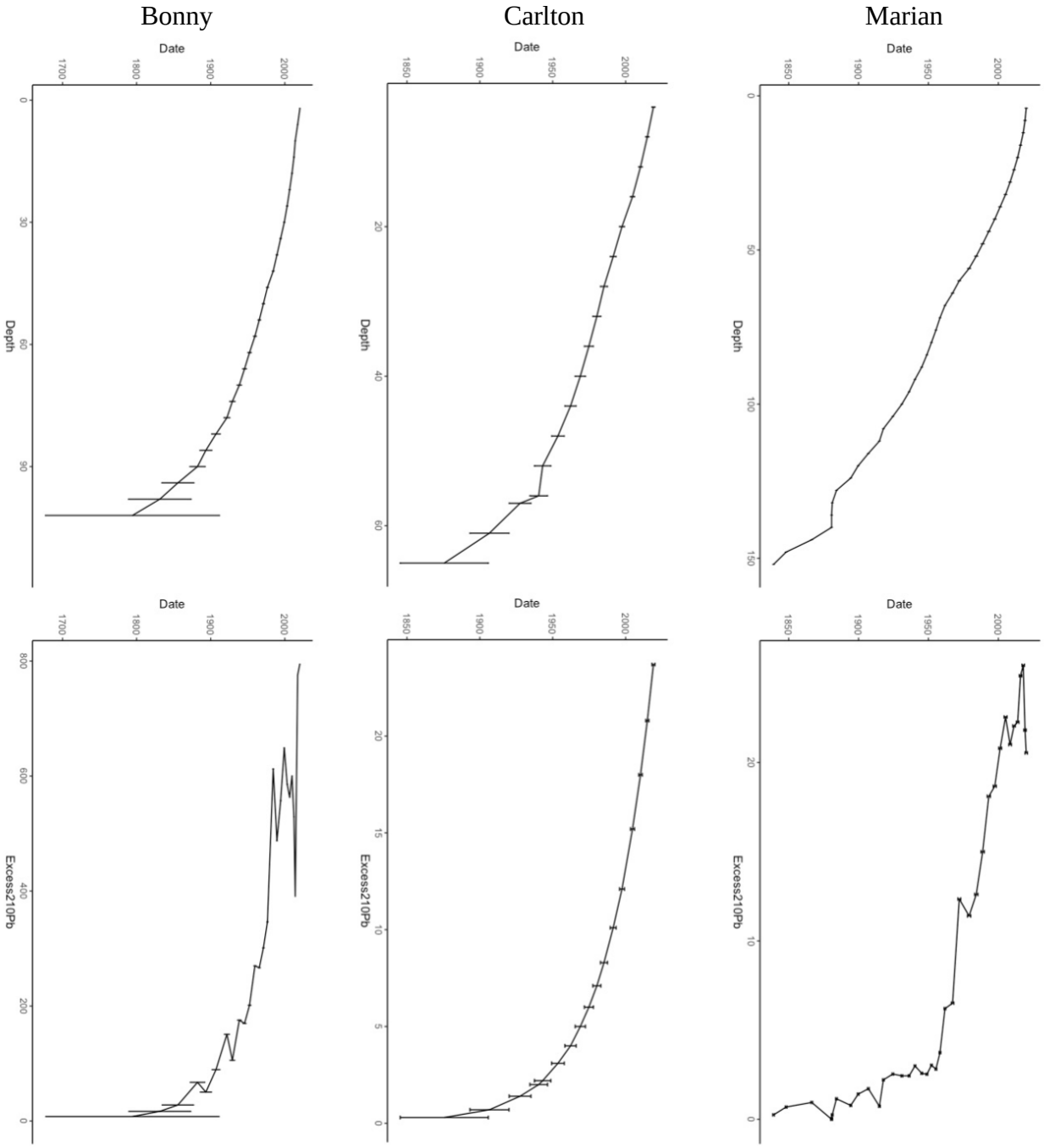


Figure 2.1: Excess $^{210}$Pb activity versus depth for each core where $^{210}$Pb dating could be utilized to establish a dating model. $^{210}$Pb activity is reported in decays per minute per gram of material.

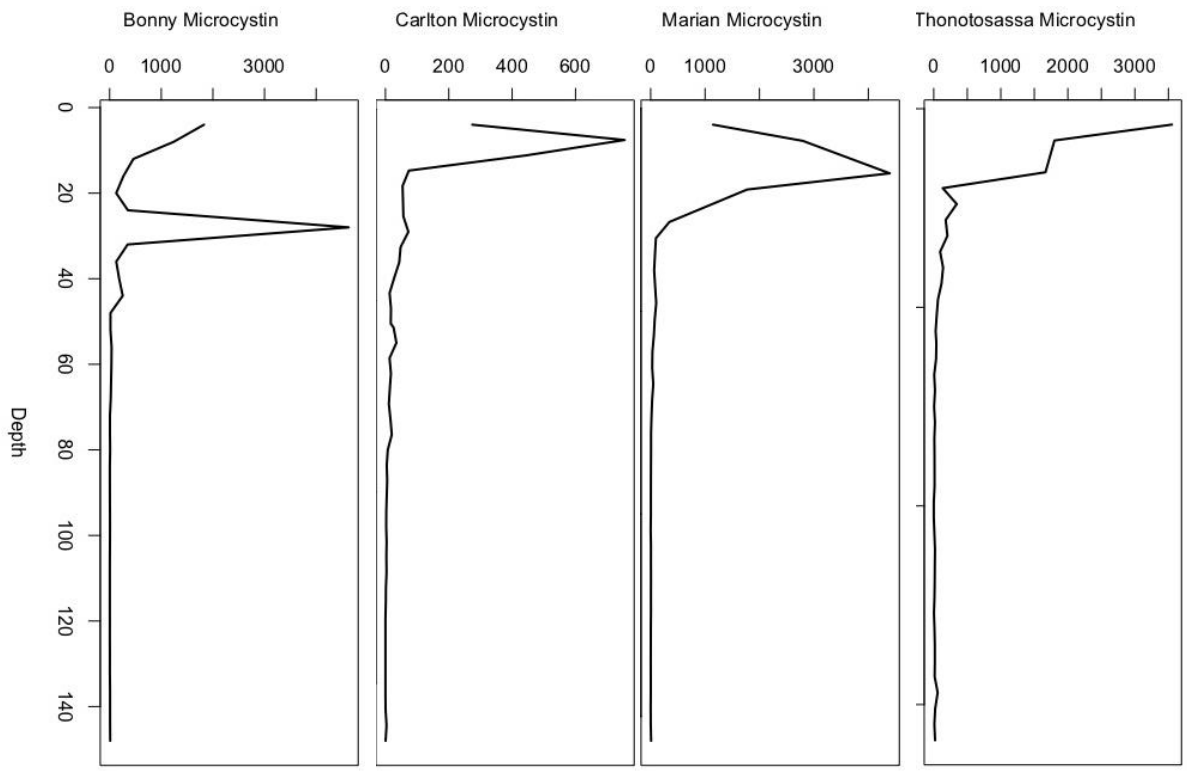


Figure 2.2: Total microcystin records versus depth for each of the study lakes reported in ng $g^{-1}$ organic matter.

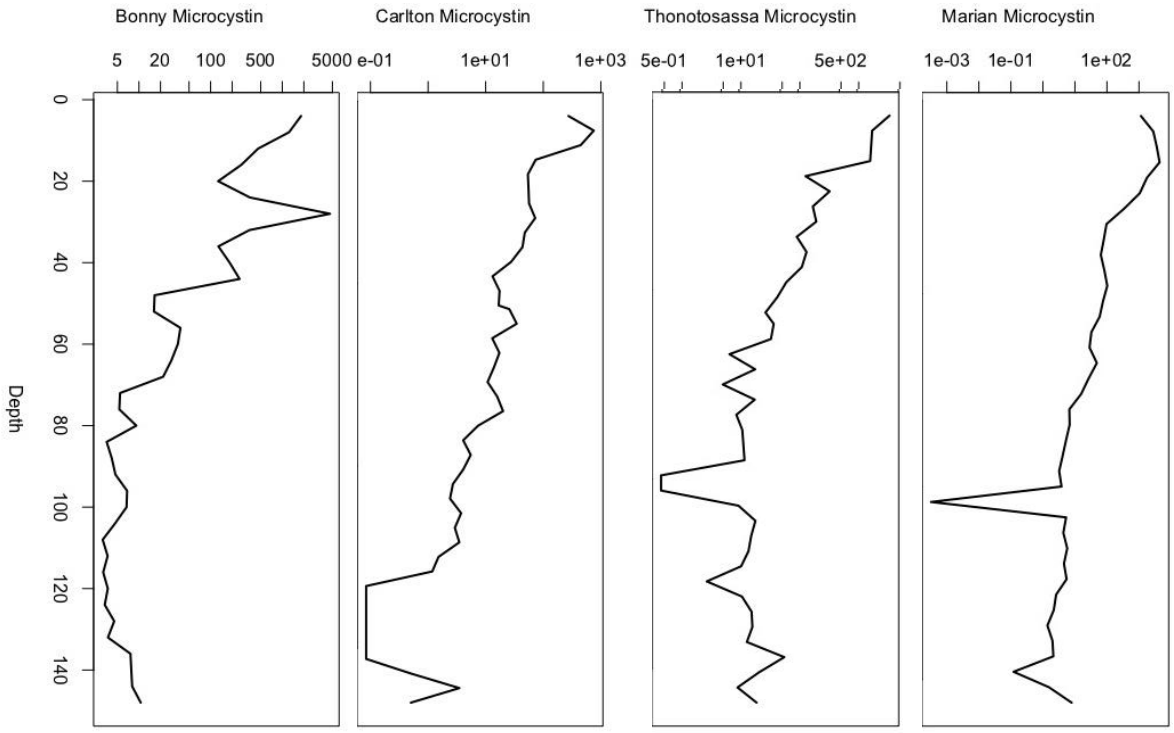


Figure 2.3: Microcystin concentrations versus depth for each of the study lakes with concentration on a log scale

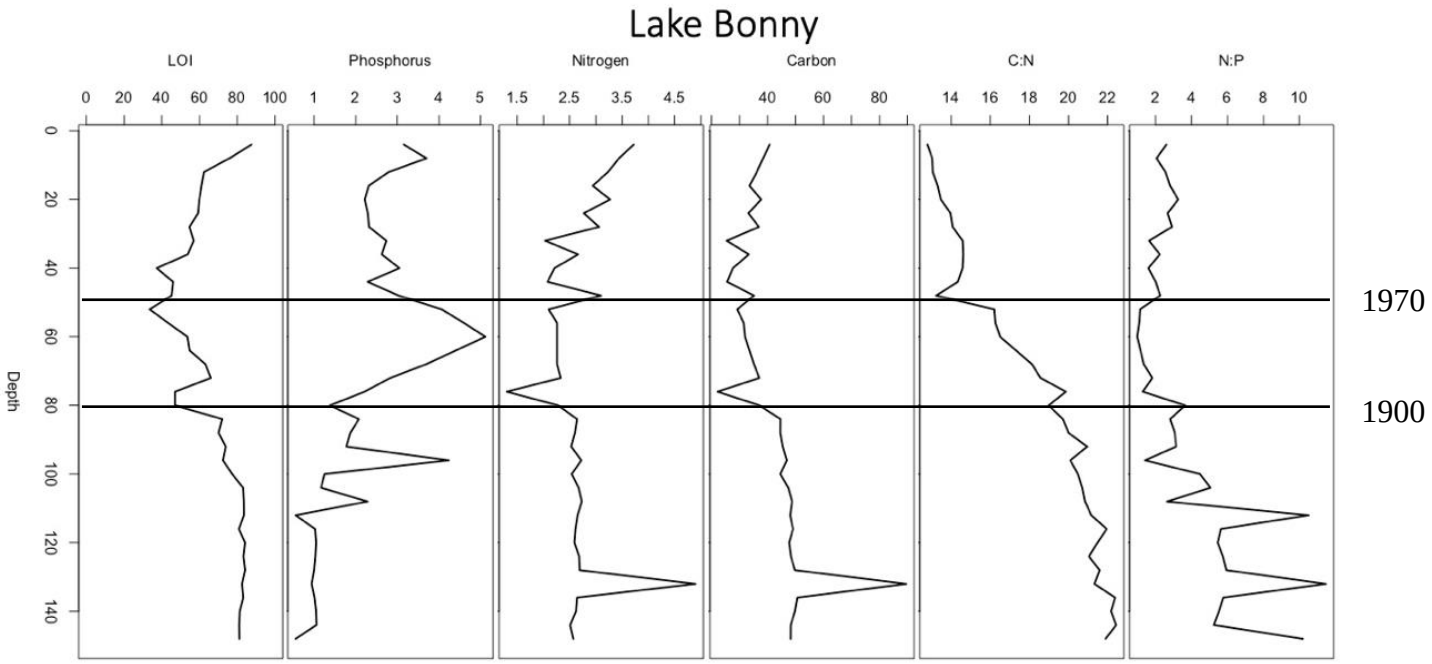


Figure 2.4: Sedimentary records versus depth for organic content (as loss on ignition), phosphorus (mg $g^{-1}$), nitrogen (%), carbon (%), carbon to nitrogen ratio, and nitrogen to phosphorus ratio versus depth for Lake Bonny. Dates are determined for excess $^{210}Pb$ measurements and the CRS model.

**2**

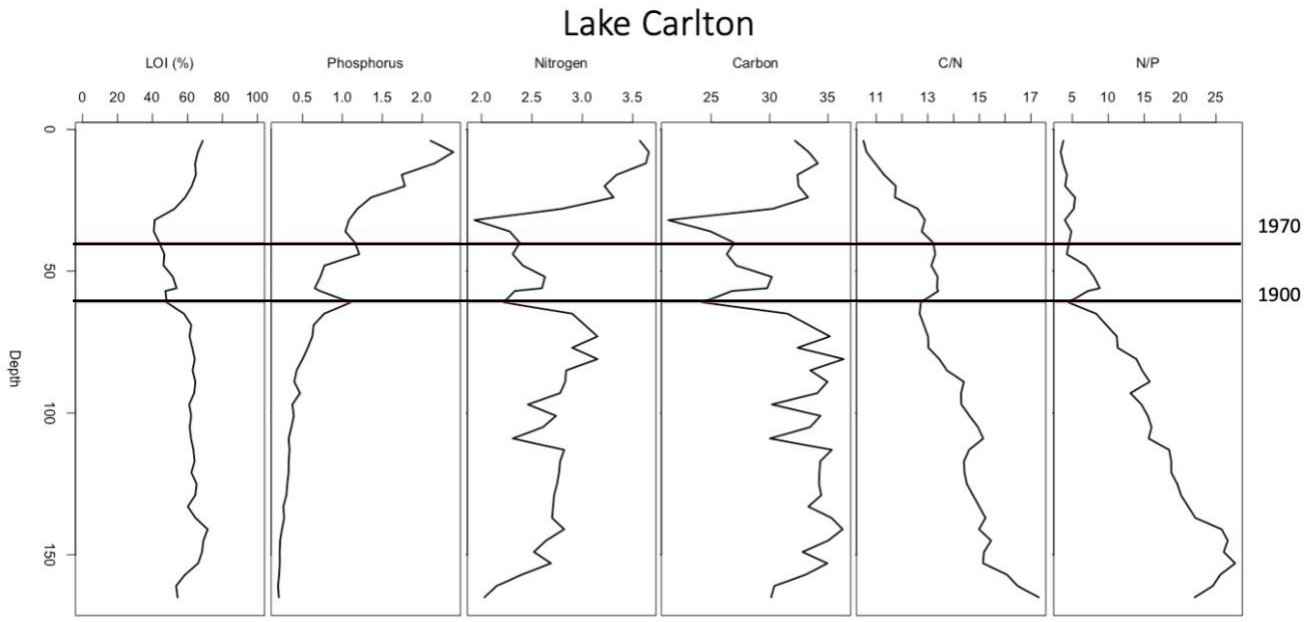


Figure 2.5: Sedimentary records versus depth for organic content (as loss on ignition), phosphorus (mg $g^{-1}$), nitrogen (%), carbon (%), carbon to nitrogen ratio, and nitrogen to phosphorus ratio versus depth for Lake Carlton. Dates are determined for excess $^{210}Pb$ measurements and the CRS model.

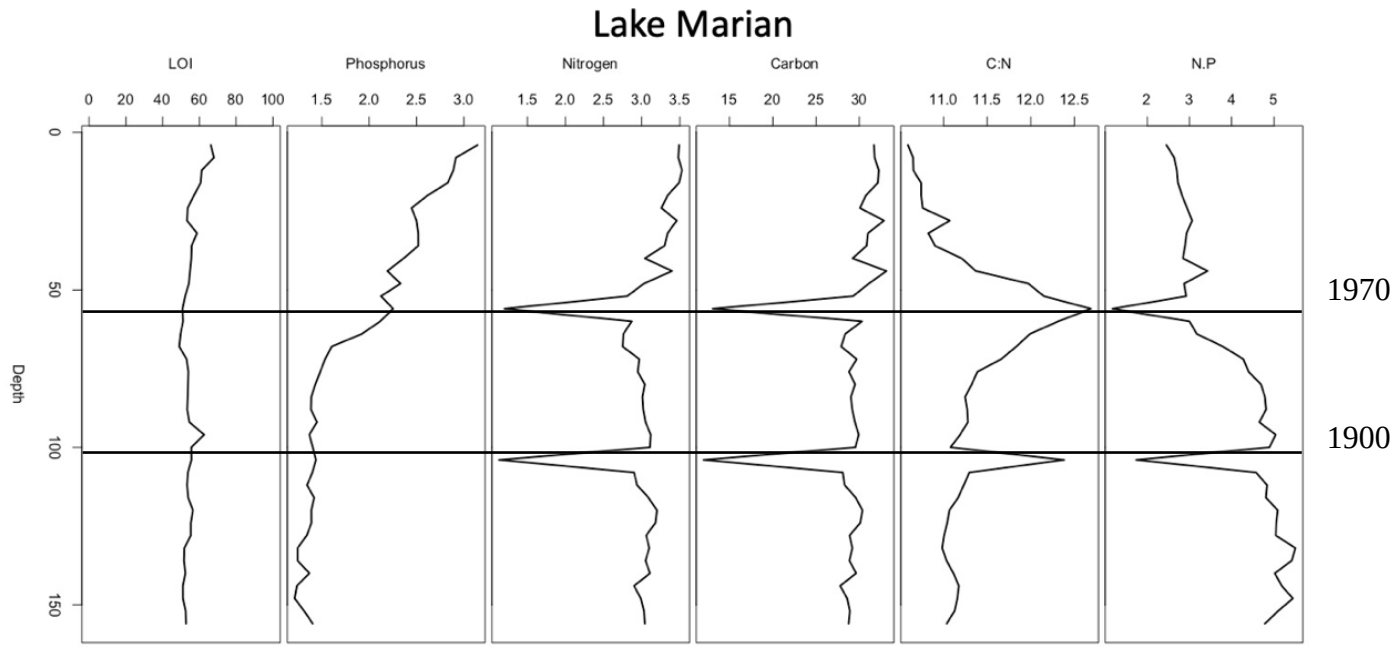


Figure 2.6: Sedimentary records versus depth for organic content (as loss on ignition), phosphorus (mg $g^{-1}$), nitrogen (%), carbon (%), carbon to nitrogen ratio, and nitrogen to phosphorus ratio versus depth for Lake Marian. Dates are determined for excess $^{210}Pb$ measurements and the CRS model.

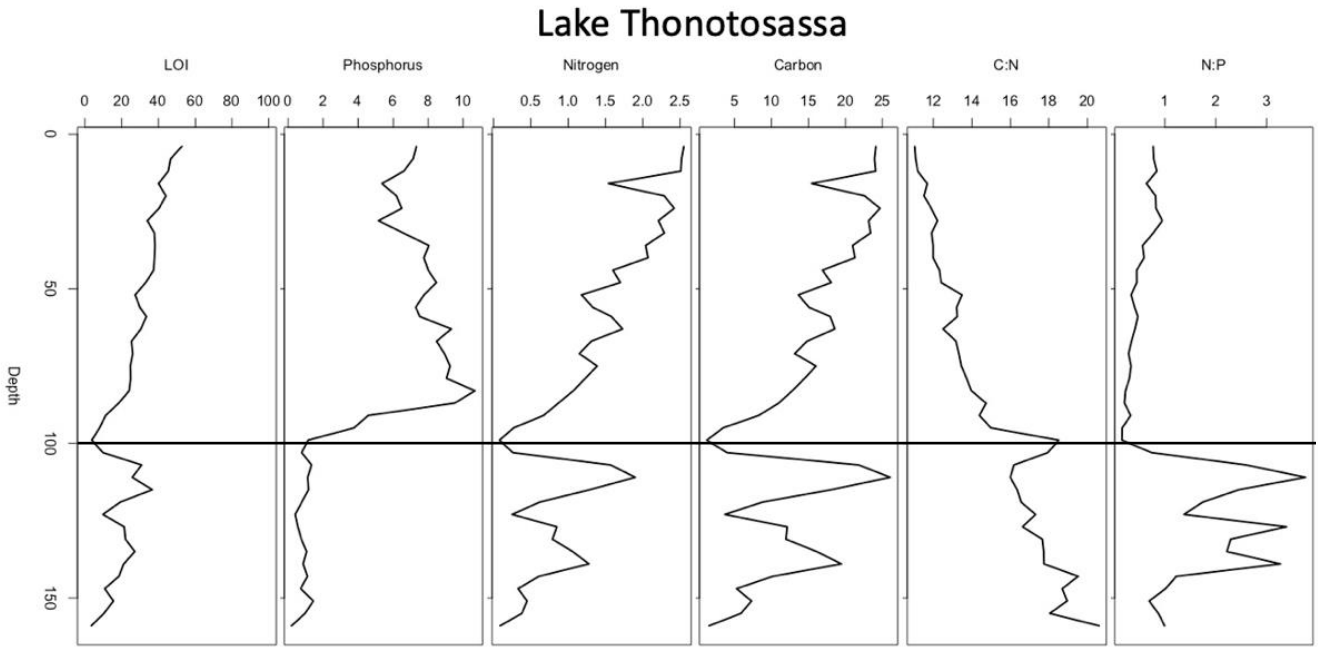


Figure 2.7: Sedimentary records versus depth for organic content (as loss on ignition), phosphorus (mg $g^{-1}$), nitrogen (%), carbon (%), carbon to nitrogen ratio, and nitrogen to phosphorus ratio versus depth for Lake Thonotosassa. 100cm depth interval is forced to 1900 per previous study (Brenner,1996).

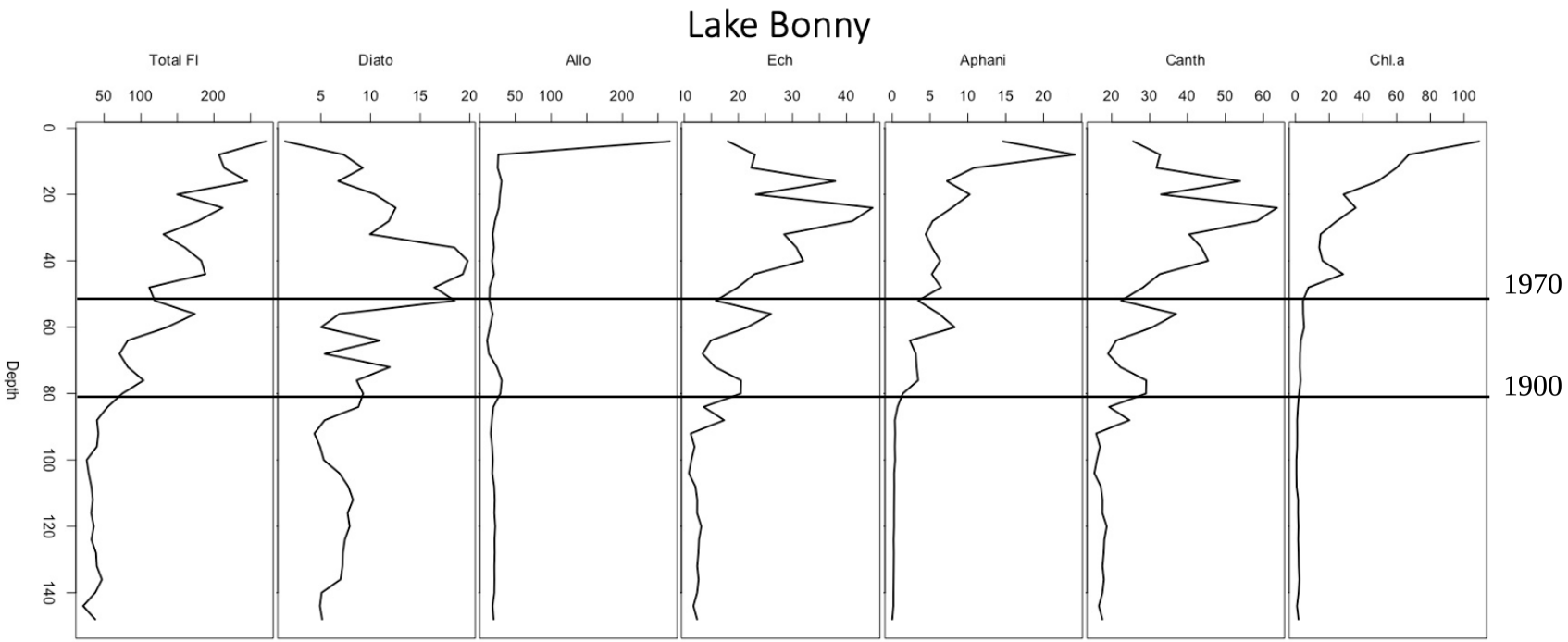


Figure 2.8: Photosynthetic pigment records versus depth from Lake Bonny: total florescence (total abundance, diatoxanthin (diatoms), alloxanthin (cryptophytes), canthaxanthin (cyanobacteria), echinenone (cyanobacteria), aphanizophyll (N2-fixing cyanobacteria), and chlorophyll a (Leavitt and Hodgson 2001). Pigments are reported in nmol pigment $g^{-1}$ organic matter. Dates are determined for excess $^{210}Pb$ measurements and the CRS model.

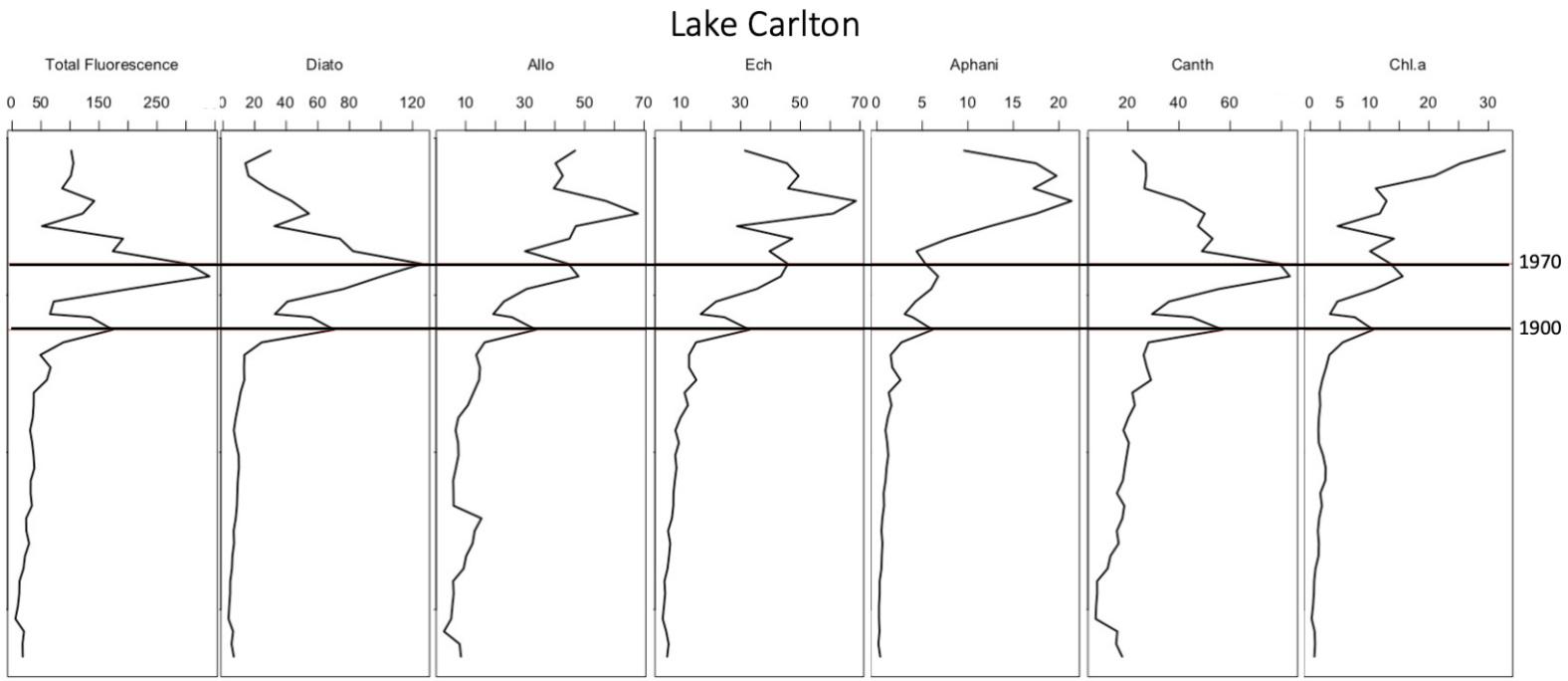


Figure 2.9: Photosynthetic pigment records versus depth from Lake Carlton: total florescence, diatoxanthin (diatoms), alloxanthin (cryptophytes), canthaxanthin (cyanobacteria), echinenone (cyanobacteria), aphanizophyll (N2-fixing cyanobacteria), and chlorophyll a (Leavitt and Hodgson 2001). Pigments are reported in nmol pigment $g^{-1}$ organic matter. Dates are determined for excess $^{210}Pb$ measurements and the CRS model.

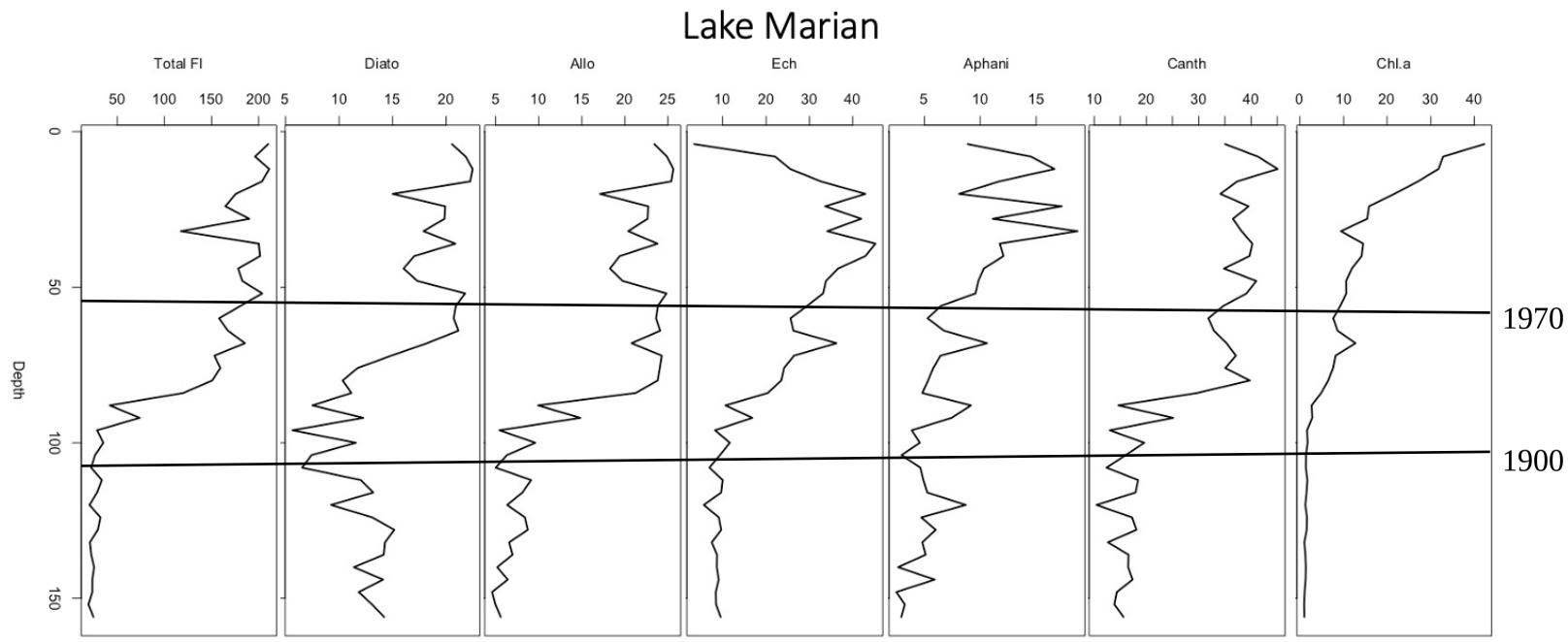


Figure 2.10: Photosynthetic pigment records versus depth from Lake Marian: total florescence, diatoxanthin (diatoms), alloxanthin (cryptophytes), canthaxanthin (cyanobacteria), echinenone (cyanobacteria), aphanizophyll (N2-fixing cyanobacteria), and chlorophyll a (Leavitt and Hodgson 2001). Pigments are reported in nmol pigment $g^{-1}$ organic matter. Dates are determined for excess $^{210}Pb$ measurements and the CRS model.

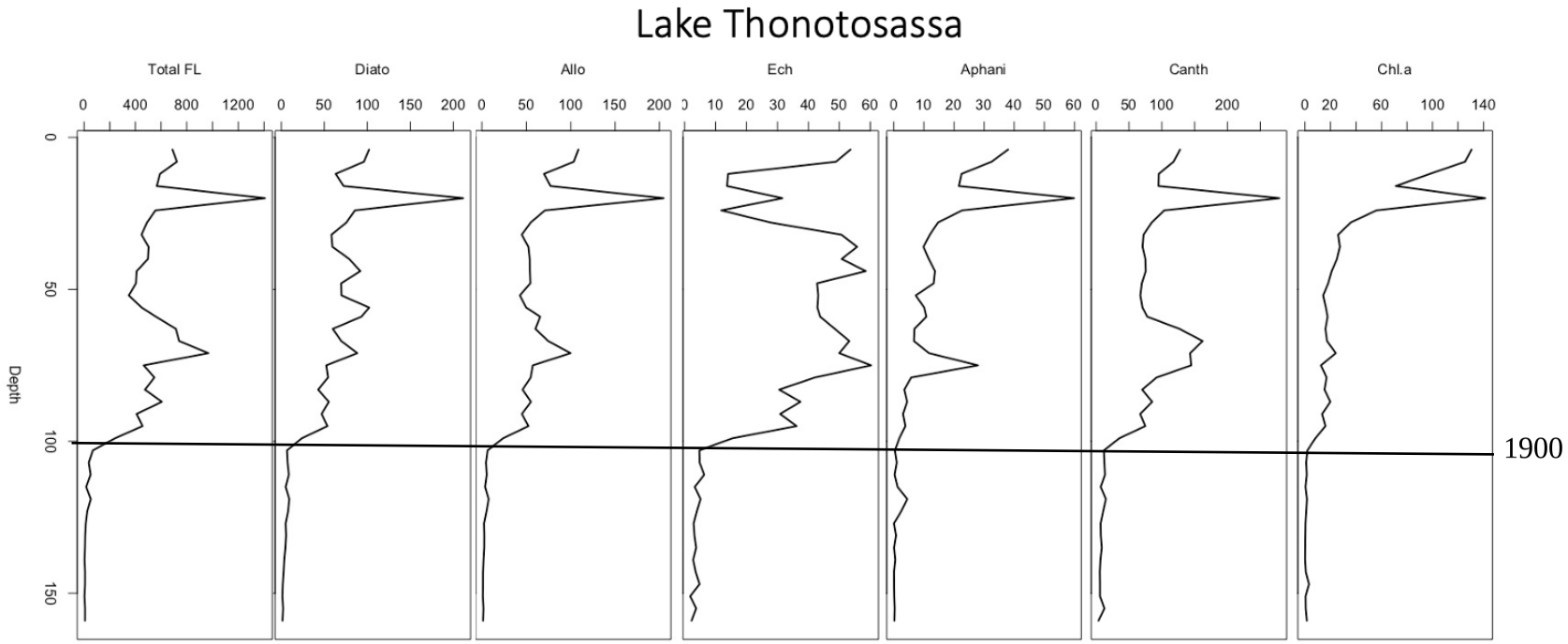


Figure 2.11: Photosynthetic pigment records versus depth from Lake Thonotosassa: total florescence, diatoxanthin (diatoms), alloxanthin (cryptophytes), canthaxanthin (cyanobacteria), echinenone (cyanobacteria), aphanizophyll (N2-fixing cyanobacteria), and chlorophyll a (Leavitt and Hodgson 2001). Pigments are reported in nmol pigment $g^{-1}$ organic matter. 100cm was forced to 1900 per previous study (Brenner 1996).

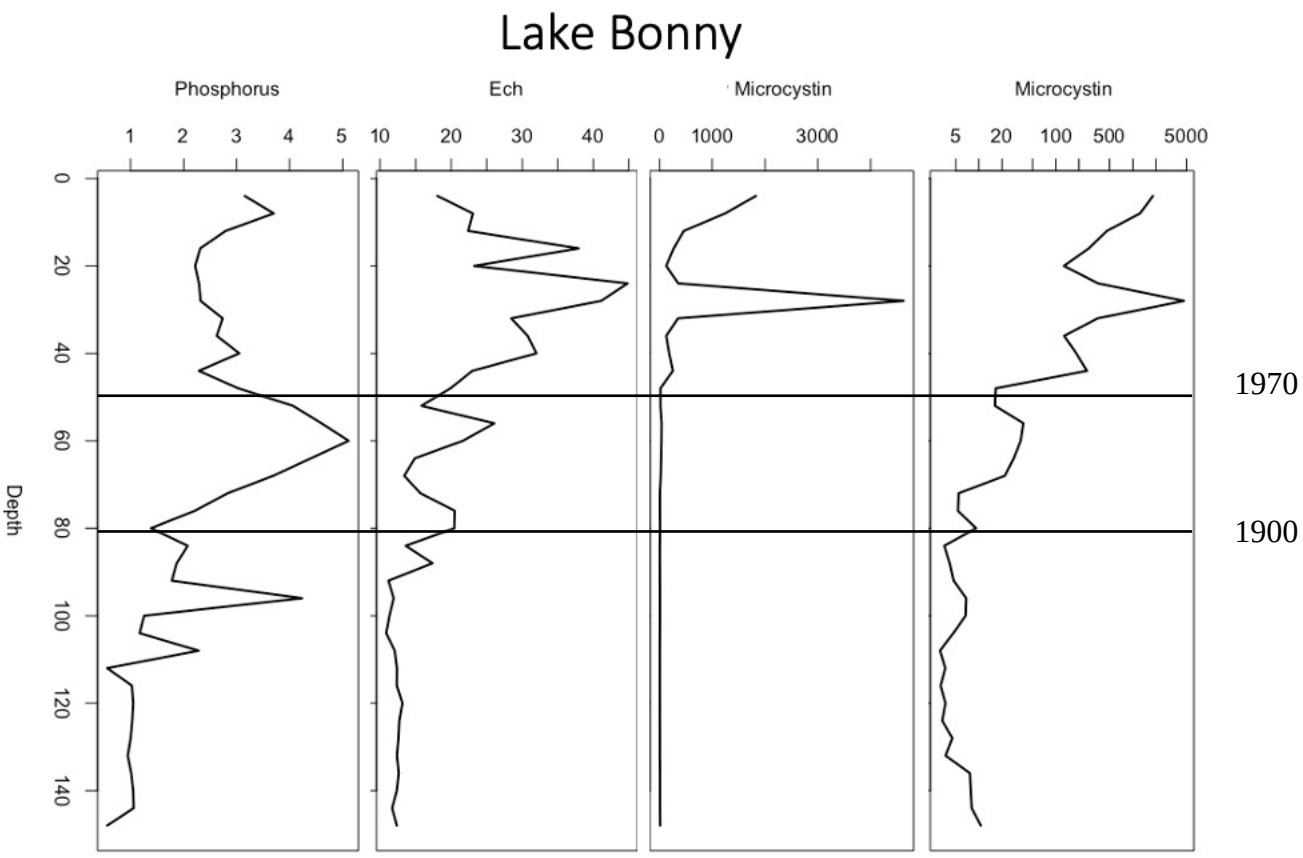


Figure 2.12: Sedimentary records versus depth of phosphorus, echinenone (Ech), and microcystin (both linear and log scaled, respectively) for the Lake Bonny core. Dates are determined for excess $^{210}$Pb measurements and the CRS model.

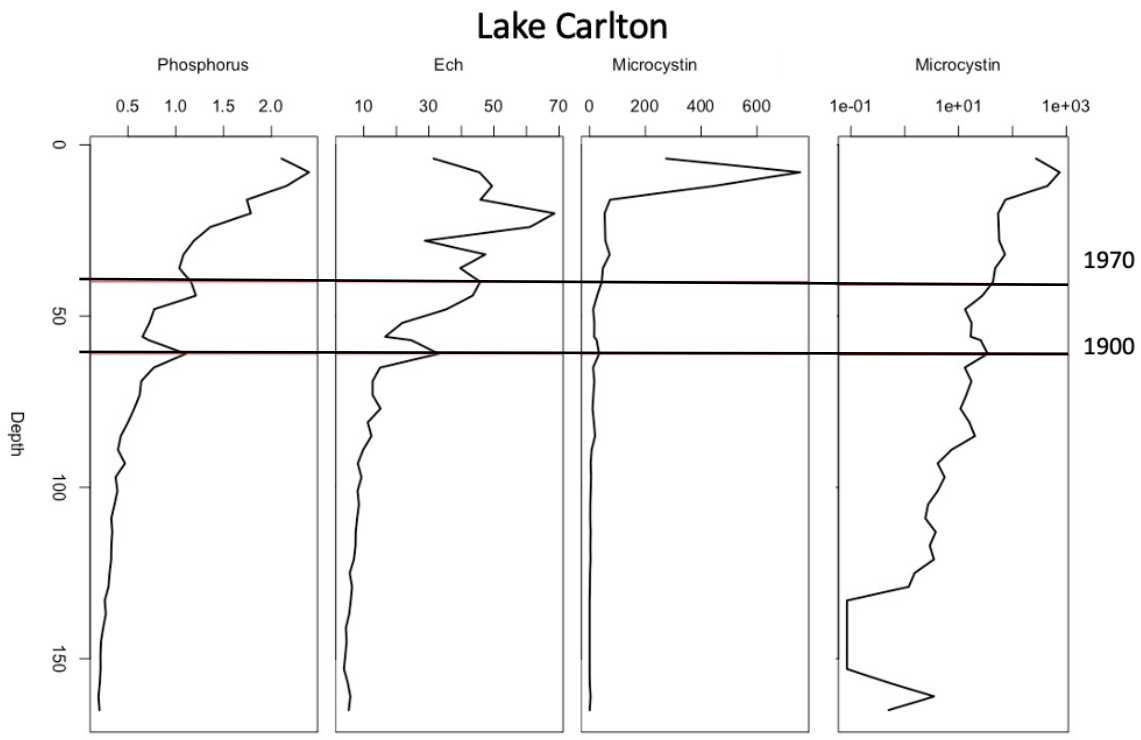


Figure 2.13: Sedimentary records versus depth of phosphorus, echinenone, and microcystin (both linear and log scaled, respectively) for the Lake Carlton core. Dates are determined for excess $^{210}$Pb measurements and the CRS model.

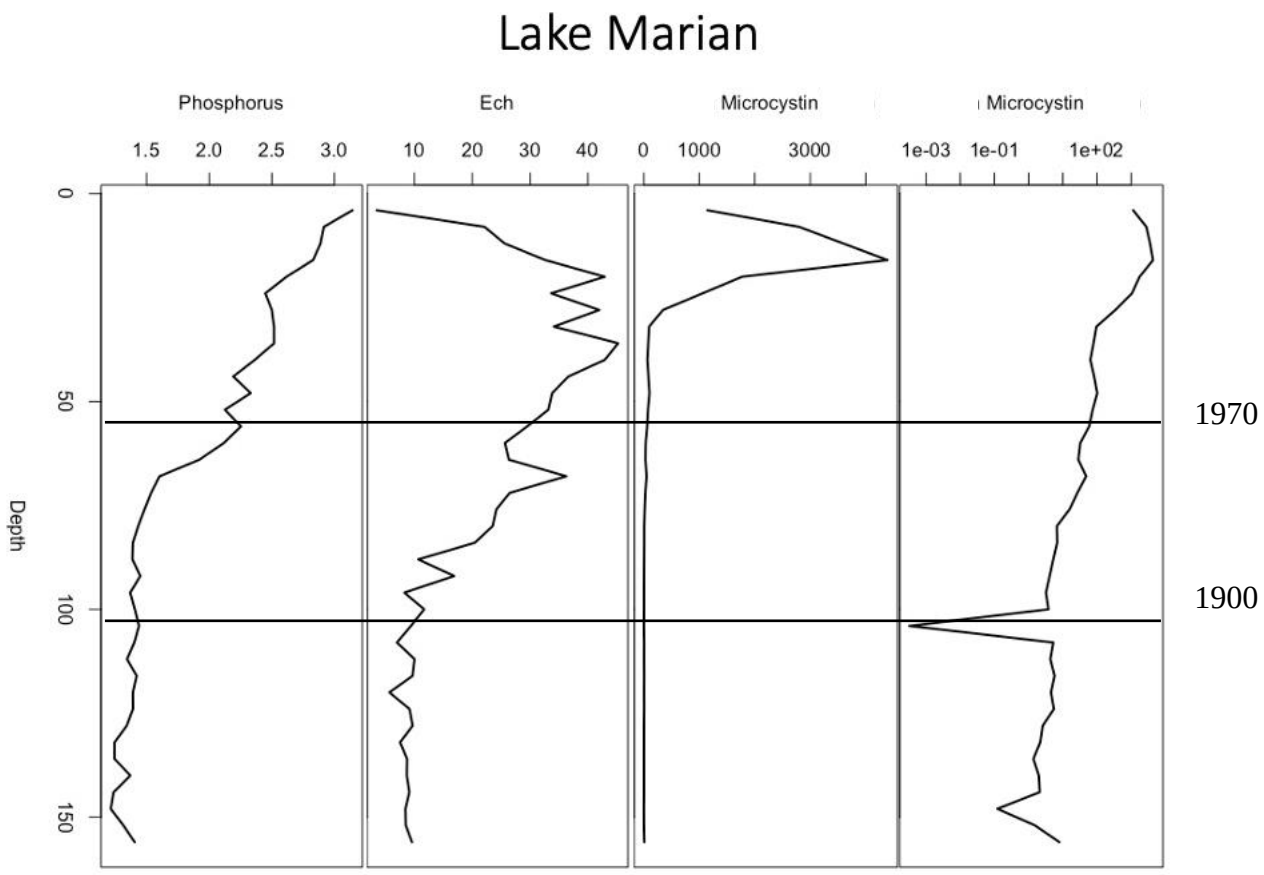


Figure 2.14: Sedimentary records versus depth of phosphorus, echinenone, and microcystin (both linear and log scaled, respectively) for the Lake Carlton core. Dates are determined for excess $^{210}$Pb measurements and the CRS model.

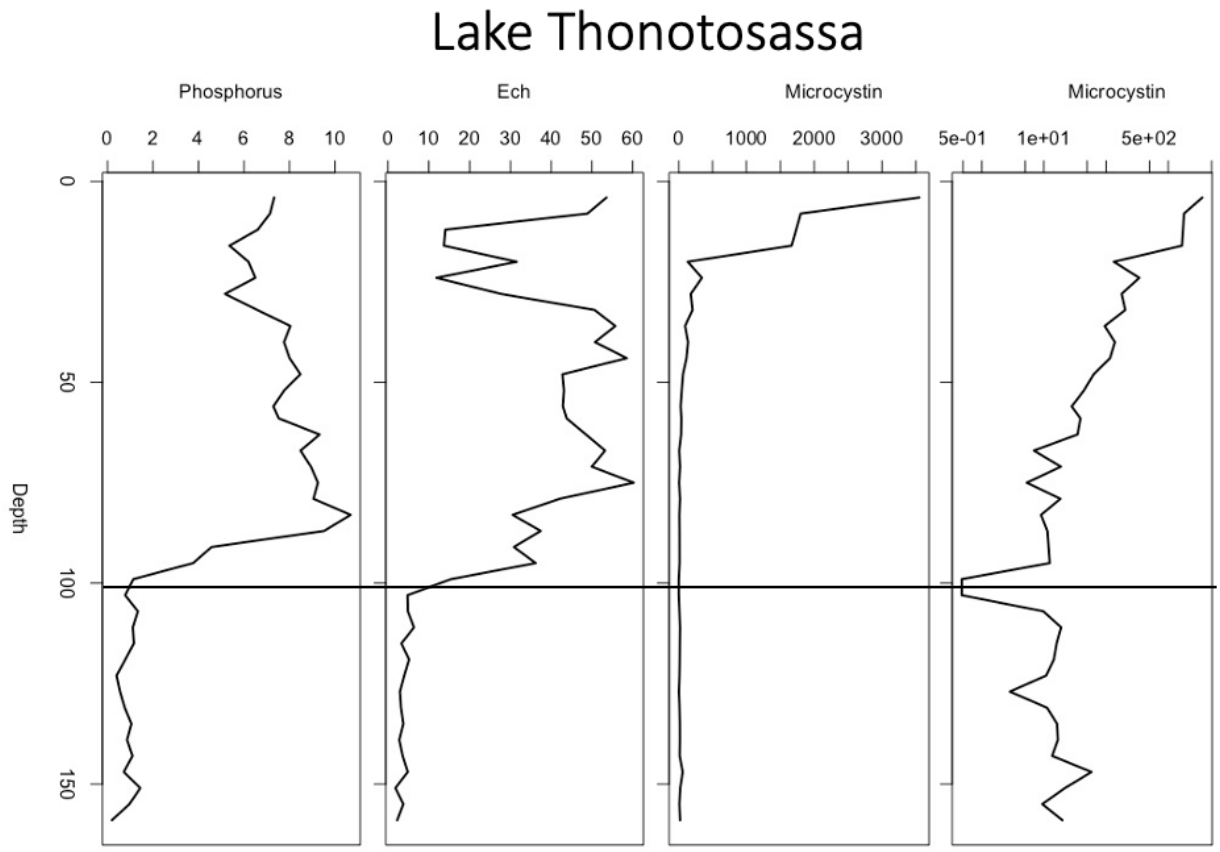


Figure 2.15: Sedimentary records versus depth of phosphorus, echinenone, and microcystin (both linear and log scaled, respectively) for the Lake Carlton core. 100cm was forced to 1900 per previous study (Brenner 1996).

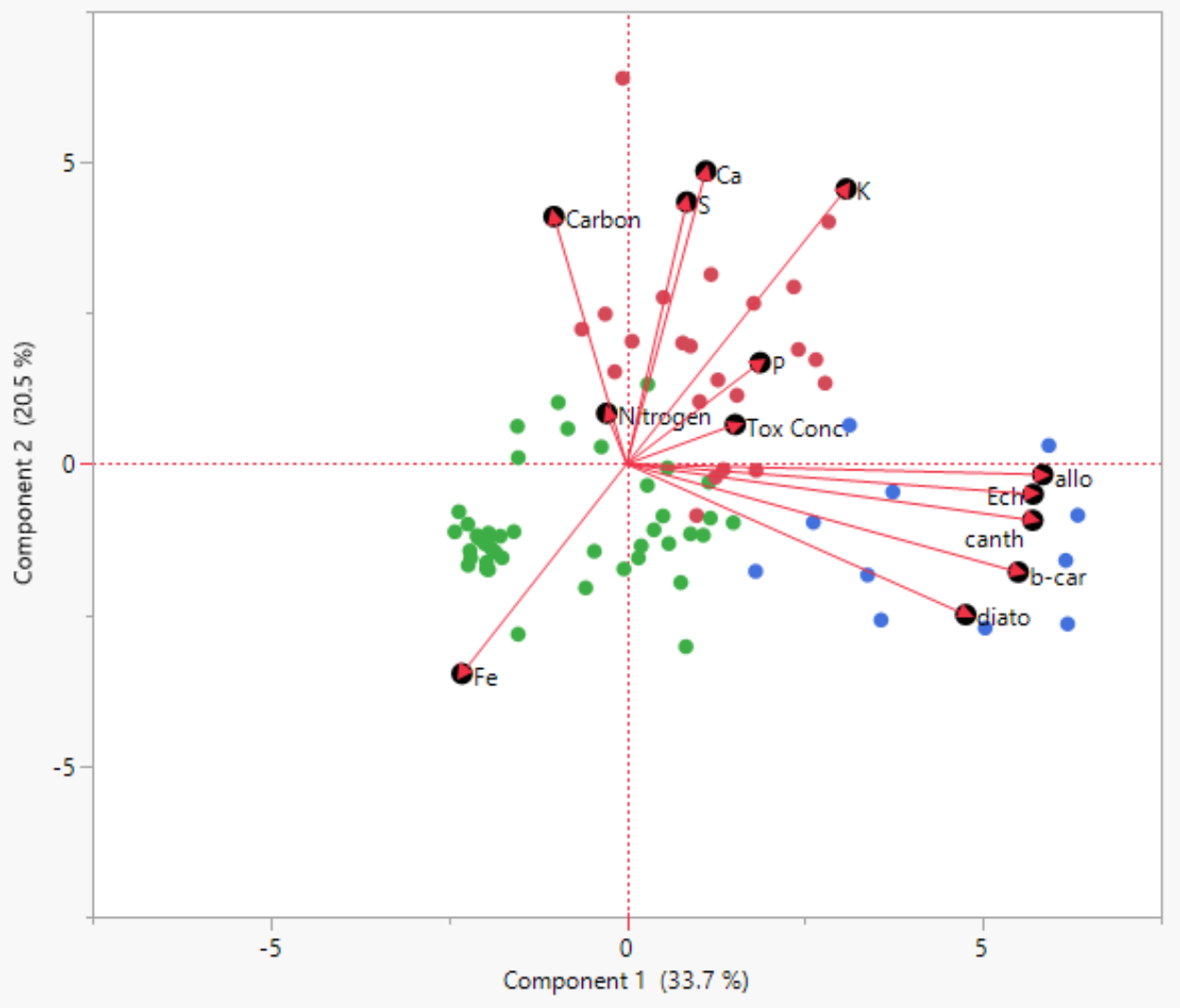


Figure 3.16: Principle component analysis from the full lake data set showing nutrients (P, C, N), photosynthetic pigments (diatoxanthin, alloxanthin, echinenone, canthaxanthin, and beta-carotene), micronutrients (Ca, Fe, K, S), and cyanotoxin concentration (Tox Conc). The samples were placed coded into groups to identify temporal relationships Pre-disturbance (green), Post-disturbance (red), and Modern (blue).

**Tables:**

Table 2.1: List of study lakes, surface areas, mean depths, dynamic ratio, and percent area subject to resuspension.

| *Lake* | *Surface Area ($km^2$)* | $Z_{mean}$ *(m)* | *Dynamic ratio* | *% Area subject to resuspension* |
|---|---|---|---|---|
| *Bonny* | 0.82 | 0.9 | 1.01 | 100 |
| *Carlton* | 1.53 | 2.4 | 0.52 | 68.8 |
| *Marian* | 23.23 | 4 | 1.21 | 100 |
| *Thon* | 3.32 | 3 | 0.61 | 78.6 |

Table 2.2: List of Study Lakes, Average Fetch (m), Average daily wind speed (m/s), wind speed needed to resuspend the mean lake depth (mph), Wind speed needed to resuspend the maximum lake depth (mph), maximum daily average wind speed recorded (mph), and maximum recorded 2-minute wind speed (mph).

| *Lake* | *Avg F (m)* | *Avg WND Sp (mph)* | *WND Sp Resuspend $Z_{mean}$(mph)* | *WND Sp Resuspend $Z_{max}$ (mph)* | *Max Daily Avg (mph)* | *Max WSF2 Recorded (mph)* |
|---|---|---|---|---|---|---|
| *Bonny* | 1300 | 6.99 | 7.83 | 26.84 | 35.12 | 121.9 |
| *Carlton* | 1320 | 7.70 | 19.01 | 31.32 | 38.7 | 79 |
| *Marian* | 2640 | 6.99 | 22.37 | 27.96 | 35.12 | 121.9 |
| *Thon* | 2500 | 6.84 | 17.90 | 26.84 | 29.08 | 81 |

Table 2.3: List of study lakes, surface areas (S.A.) ($km^2$), microcystin loads (ug), microcystin loads (Kg), lake volume (L), 50% lake bed resuspension water column microcystin concentration (ug $L^{-1}$), full lake bed resuspension water column microcystin concentration (ug $L^{-1}$), and the number of days per year exhibiting wind velocities capable of resuspending the full lake bed.

| *Lake* | *S.A. ($km^2$)* | *MYC load (ug)* | *MYC Load (Kg)* | *Volume (L)* | *50% Resuspension (ug $L^{-1}$)* | *Full Resuspension (ug $L^{-1}$)* | *Full Resuspension (days/year)* |
|---|---|---|---|---|---|---|---|
| *Carlton* | 1.53 | 1.368E+10 | 13.68 | 7.45E+9 | 0.92 | 1.84 | 16 |
| *Thon* | 3.5 | 8.973E+11 | 897.37 | 1.12E+10 | 40.00 | 80.01 | 9 |
| *Marian* | 23.23 | 2.363E+12 | 2363.65 | 5.78E+10 | 20.46 | 40.93 | 16 |
| *Bonny* | 0.82 | 8.33E+8 | 0.83 | 1.77E+9 | 0.24 | 0.47 | 9 |

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Chapter 3: Paleolimnological Evidence for primary producer change in Lake Carlton due to Hydrologic Connectivity and Human Impacts

**Abstract:**

Lake hypereutrophic conditions are generally associated with nutrient inputs from surrounding terrestrial landscapes. However, some systems can receive primary nutrient inputs through hydrologic connections such as rivers or canals. Lake Carlton, Florida, USA is a small, shallow, polymictic lake that forms a hydrologically connected string of lacustrine systems with hypereutrophic Lakes Beauclair and Apopka. Lake Beauclair and Lake Apopka were connected hydrologically in 1893 Common Era (CE) and have maintained excessive hypereutrophic conditions despite extensive management to reduce nutrient inputs. Here, I collected a sediment core from Lake Carlton to answer two primary research objectives: 1) to reconstruct the nutrient input and storage for Lake Carlton throughout the last 200 years to see if nutrient inputs result from hydrologic connections or local terrestrial sources, and 2) to link primary producer change with management actions between Lakes Apopka, Beauclair and Carlton. Paleolimnological tools were applied to a 165-cm core taken from the depositional area of the lake and analyzed for bulk density, organic matter content, nutrients (C, N, P), photosynthetic pigments, and total microcystins. Sediments were dated using $^{210}$Pb and results indicate that the core represents over 100 years of sediment accumulation. Nutrient deposition shows that the primary driver of nutrient inputs resulted from the Apopka-Beauclair connection in 1893 CE with less impact from terrestrial inputs. Photosynthetic pigment data indicate a pattern of eutrophication coinciding with the hydrological modification as well. However, around ~1970 CE, primary producer communities shift from diatom dominance to cyanobacteria dominance, which appears to be

linked to more local impacts on the lake ecosystem. Cyanotoxin production records, however, show a significant lag between regime shift and peak cyanotoxin production. These data demonstrate that local nutrient inputs do not govern all phytoplankton dynamics in shallow lake systems but must be interpreted in light of hydrologic alterations and management.

**Introduction:**

Since the industrial revolution, anthropogenic impacts to aquatic ecosystems have accelerated nutrient loading, modified terrestrial landscapes, and altered the global climate (Steffen 2007). For lake ecosystems the acceleration of nutrient loading from agricultural activities and urbanization have increased net primary production and hypereutrophic conditions (Schindler 1977). Increased surface water temperatures caused by global climate change can strengthen thermal stratification and lengthen optimal growing periods for phytoplankton as well as favor cyanobacteria growth and dominance (Paerl 2008). Furthermore, the construction of canals can cause hydrologic alterations lowering lake levels and increasing nutrient and sediment loading (Waters et al. 2009). Intensified land use, sewage discharge, and climate change during the past two centuries will promote the growth and proliferation of harmful algae in freshwaters (Wolfe 2013).

As lakes experience increased eutrophication due to cultural impacts on watersheds and lake ecosystems, cyanobacteria are given a competitive advantage over other phytoplankters (Heisler, 2008). Cyanobacteria are less nutritious (Robidoux et al. 2015) for zooplanktonic predators, which can cause a negative bottom-up effect on higher trophic levels. Fish and invertebrates lose habitat through a reduction in the number and diversity of aquatic plants and the lack of nutritional value from cyanobacteria (Robidoux et al. 2015). This increase in cyanobacteria abundance can confound ecosystem services through anoxia, biological stress, and

the production of toxins which can be hazardous to human health (Paerl 2008). Whereas nutrients are considered the primary drivers of phytoplankton dynamics in most lakes, additional stressors such as hydrologic change, internal nutrient loading, temperature increases, and fish management have also been shown to increase cyanobacteria abundance. As lakes begin to change due to these stressors, understanding how multiple drivers may impact primary producer community structure and ecosystem function are key for managers to make informed decisions.

While nutrient inputs from terrestrial landscapes have been the focus of the majority of lake eutrophication, recent studies have shown that hydrology can also be a driver of eutrophication and primary producer increase. Finlay et al. (2013) showed that hydrologic residence time related to primary producer growth and N dynamics for a multi-lake dataset focused primarily on northern temperate lake ecosystems. Webster et al. (2021) showed that reservoirs connected in sequence can influence nutrient dynamics differently with P related to the placement of the aquatic system in the reservoir sequence and N related to hydrologic residence time. Further research has shown that hydrologic alterations and connection through canals can cause cyanobacteria dominance both through decreases in water level (Waters et al. 2009) and increases in water level (Waters et al. 2015). Whereas total nutrient inputs are of importance, these studies collectively demonstrate that hydrology can impact nutrient dynamics post-input and subsequently impact primary producer communities.

Here, I collected a sediment core from Lake Carlton, Florida, USA to investigate the impacts of nutrient inputs, hydrologic alterations, and other stressors on primary producer community structure. Lake Carlton is connected to Lakes Beauclair and Apopka, which form a string of lakes with a distinct nutrient source in Lake Apopka and known dates for hydrologic connectivity. Paleolimnological tools applied to the core were bulk density, organic matter,

nutrients (C, N, P), photosynthetic pigments, and cyanotoxins. The primary research objectives were: 1) to reconstruct the nutrient input and storage for Lake Carlton throughout the last 200 years to see if nutrient inputs result from hydrologic or local terrestrial inputs, and 2) to compare primary producer change in response to management actions between Lakes Apopka, Beauclair and Carlton.

**Methods:**

*Study Site:*

The Harris Chain of Lakes in central Florida was subject to large increases in anthropogenic nutrient loading and hydrologic modification beginning in 1893. After a series of canals strengthened hydrologic connectivity between the lakes, poor agricultural practices around Lake Apopka lead to widespread nutrient pollution throughout the Harris Chain (Schelske et al. 2005). Increased nutrient loading in Lake Apopka had downstream effects causing increased P concentrations and organic matter deposition in Lake Beauclair (Schelske et al. 2005). Due to the direct hydrologic connection to Lake Beauclair via the "Carlton Cut", Lake Carlton was changed from an isolated lake within the watershed to a connected lake within the larger Harris Chain system. The Florida Department of Environmental Protection has theorized that P-rich surface waters are pushed from Lake Beauclair to Lake Carlton by wind-driven water movement (Amec 2018). Previous work used sediment core analysis to determine the downstream effects Lake Apopka has had on other members of the Harris Chain of Lakes (Schelske et al. 2005). These methods could be applied to sediment records obtained from Lake Carlton to extrapolate nutrient pollution sources and compare eutrophication histories to a lake that has been altered from an isolated to a connected state.

Lake Carlton is a 1.53 $km^2$ public lake situated between Orange County and Lake County, Florida, USA (28°45'34.9"N 81°39'27.0"W) (Fig 3.1). Lake Carlton is connected to Lake Beauclair via the Carlton Cut but is not directly connected to any other lakes in the Harris Chain. Listed as "impaired" by the Florida Department of Environmental Protection in 2002, Lake Carlton is a hypereutrophic, solution depression lake currently dominated by cyanobacteria with Trophic Status Index values ranging from 46-94, phosphorus concentrations ranging from 6 ug $L^{-1}$ to 760 ug $L^{-1}$, and chlorophyll-a concentrations ranging from 0.6 ug $L^{-1}$ to 302 ug $L^{-1}$. TSI values, nutrient concentrations, and pigment concentrations have decreased in recent years (Amec 2018).

*Sample Collection:*

A sediment core was collected during the summer of 2020 using a piston-coring device that maintains an undisturbed sediment-water interface (Fisher et al. 1992). The core was collected in two sections with each section sampled in the field at 4-cm intervals. Sediment samples were stored on ice and in the dark until they were returned to the lab at Auburn University. Sectioning at 4-cm intervals, as opposed to smaller sections, was based on previous paleolimnological investigations in Florida (Brenner et al. 1999, Schelske et al. 2005, Waters et al. 2005) and to allow for enough sediment material for analytical measurements. Sediments from hypereutrophic systems in Florida are subject to wind-driven resuspension (Bachmann 2000), which can lead to uneven sediment distribution within the basin (Whitmore et al. 1996). Based on information from previous studies and bathymetric data, areas likely to facilitate sediment accumulation were chosen for coring locations.

*Laboratory Analysis:*

A wet aliquot of known volume was removed from each sample for bulk density and gravimetric analysis. Organic matter content and non-volatile solids were determined through loss on ignition (LOI) at 550°C in a muffle furnace for 3 hours and reported as a percent (Hakanson and Jansson 1983). Samples were then frozen, freeze-dried, and ground with a mortar and pestle for further analysis. Carbon and nitrogen were analyzed using a Costech Combustion Elemental Analyzer with an attached autosampler. For organic carbon, samples were acidified in HCl vapor for 24 hours prior to analysis. For additional elements (P, Fe, S and other elements), sediment samples were analyzed using ICP-ARL following acid digestion in a heated block following EPA 6010B.

Fossilized photosynthetic pigments were measured using a high-performance liquid chromatography system (HPLC) following the methods of Leavitt and Hodgson (2001) and Waters (2016). Freeze-dried sediment samples were extracted with a solvent mixture of acetone, methanol, and water (80:15:5), which contains an internal standard (Sudan II: Sigma Chemical Corp.). Extraction was done for 16-24 hours in a -20$^{\circ C}$ freezer. Samples were injected into the Shimadzu HPLC system following the mobile phase and time sequence of Leavitt and Hodgson (2001). Chlorophylls and carotenoids were separated by passing through a Phenomenex Luna C18 column and measured using a photodiode array detector coupled with a fluorescence detector. Pigments were identified using retention times and pigment-specific spectra of known standards. To compare chlorophylls and carotenoids, all pigments are reported as nmol $g^{-1}$ org. matter.

Total microcystins were measured using the total microcystin/nodularin ADDA enzyme-linked immunosorbent assay (ELISA) kit commercially available from Eurofins (abraxis.eurofins-technologies.com). Total microcystins were extracted from dried sediment

samples by combining the methods provided by Eurofins for soils and from a previous study (Zastepa et al. 2017). A mixture of 75% methanol and 25% water acidified with trifluoracetic acid, was used as the extraction solution. 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 with the ELISA kits.

Samples for radiometric dating were packed in plastic test tubes, sealed with epoxy, and stored for >21 days in order for samples to reach $^{226}Ra/^{210}Pb$ equilibrium. An ORTEC Intrinsic Germanium Detector was used to measure $^{210}Pb$, $^{214}Bi$ (i.e. $^{226}Ra$), and $^{137}Cs$ activity by direct gamma counting (Appleby and Oldfield 1986). $^{226}Ra$ activity is used as a proxy for "supported" $^{210}Pb$ activity—the activity generated by *in situ* radium in the sediments. "Unsupported" $^{210}Pb$ activity is calculated as the difference between total $^{210}Pb$ activity and supported $^{210}Pb$ activity. Atmospheric deposition of $^{210}Pb$ occurs at a constant rate and, assuming that the lake morphology remains constant over time, can be used to assign dates to sediment samples through the Constant Rate of Supply Model (CRS) (Appleby and Oldfield 1983).

**Results:**

The 165-cm sediment core consisted of dark organic sediments throughout the core with highly flocculent sediments in the top 16 cm. Excess $^{210}$Pb steadily decreased down core and the CRS provided dates for the top 64-cm of the core spanning the last ~100 years and showed that the entire Lake Carlton sediment record spans >150 yrs (Fig. 3.1). This dating model was further validated by similar stratigraphic changes in non-volatile solids between the Lake Carlton and Lake Beauclair (Schelske et al. 2005) cores (Fig. 3.2).

In Lake Carlton, a sharp increase in P was observed ~1900 Common Era (CE) (61 cm) followed by a steady increase to the top of the core. Maximum P storage was 2.4 mg $g^{-1}$ in 2015. N had a maximum of 3.66 % around 2015, with a potential outlier of 5.21% predating the $^{210}$Pb record at 76 cm. Maximum C concentrations of 36.25% also predate the $^{210}$Pb record at a depth of 84 cm. Nutrient ratios exhibited shifts with N:P values before anthropogenic nutrient loading ranging from 27.6 to 9.7 before 1900 and then from 8.3 to 3.7 after excess P is introduced (Fig. 3.3). C:N experienced a steady decline throughout the core with historic values decreasing from 17 to 12.6 before 1900 and decreasing from 12.7 to 10.5 after anthropogenic impacts to the top of the core (Fig. 3.3).

All photosynthetic pigment records indicate phytoplankton abundance increased throughout the record with a sharp spike in productivity that corresponds with increases in P at 61 cm or ~1900 CE (Fig 3.4). Following this increase, all photosynthetic pigments and total fluorescence spiked at 61 cm. Chl-a showed peaks at 61 cm and 44 cm with the highest values in the top of the core. Diatoxanthin reached its maximum concentration at 40 cm and decreased to lower levels from 1970 to the present day. Alloxanthin had several peaks (61 cm, 44 cm) including a maximum at 24 cm in ~1992. Aphanizophyll and Echinenone exhibited similar peaks

and troughs throughout the core and both reached maximums at 20 cm in ~1997. Canthaxanthin followed similar trends to Diatoxanthin as it reached its maximum concentration at 40 cm and decreased to lower levels following 1970 to the present day. PPCS shifted towards the top of the core, moving from diatom dominance to cyanobacterial dominance after 1970 at a depth of 36 cm (Fig. 3.4) as indicated by stratigraphic change from diatoxanthin (diatoms) and aphanizophyll (cyanobacteria). Low amounts of microcystin were detected through the core with a large increase in recent sediments corresponding to ~2005 CE (3.6). Detectable amounts of microcystin were present before large-scale, human disturbance at 85 cm.

Previous work from Schelske et al. (2005) was used to corroborate our dating model and stratigraphic changes within the Lake Carlton core. The Schelske et al. (2005) study used sediment core analysis to connect nutrient loading in Lake Beauclair to the excess nutrients in Lake Apopka. Stratigraphic changes in non-volatile solids, P, and C:N ratios showed Lake Apopka has a direct influence over the nutrient dynamics within Lake Beauclair. I compared their data to the core recovered from Lake Carlton (Figure 3.7). Both Lake Carlton and Lake Beauclair exhibit similar peaks in non-volatile solids ~1950, as well as slight decreases in P and increases in C:N ratios. Through these similarities in stratigraphic changes in non-volatile solids, P, and C:N ratios, I can demonstrate direct influences from Lake Beauclair and Lake Apopka on Lake Carlton.

**Discussion:**

The sediment record from Lake Carlton demonstrates the increased P loading and subsequent increase in algal production coincided with the Apopka-Beauclair Canal's construction in 1893. As a result, the P inputs impacting Beauclair from Apopka (Schelske et al. 2005) further impacted Carlton on a very similar timescale. Schelske et al. (2005) used non-

volatile solids data from Lake Apopka and Lake Beauclair to demonstrate the hydrologic connectivity between these two lakes in order to construct a dating model for Lake Apopka. The sediment record from Lake Carlton exhibits the same trends in non-volatile solids validating P source assumptions that nutrient inputs entering Lake Carlton originated from Lake Apopka. These hydrologic connections between Lake Carlton and Lake Beauclair have been documented by the Florida Department of Environmental Protection and the Lake County Water Authority (Amec 2018) through monitoring efforts, but my data show the long-term temporal connection following canal construction.

Total primary producer abundance and diagnostic photosynthetic pigment concentrations suggest several periods of increased primary producers throughout the core in response to both P originating from Lake Apopka and more recent change in phytoplankton community structure. The first of these peaks coincides with the construction of the Apopka Beauclair Canal in 1893 (Fig. 3.4) and corresponded to an increase in all pigments demonstrating a dominance by diatoms (diatoxanthin) and some cyanobacteria (canthaxanthin). After ~1930, pigment concentrations briefly decrease then increase to form another peak around ~1970. The 1970 fossil pigment peak indicated changes in PPCS from diatom dominance to cyanobacterial dominance. These variations in pigment concentrations correspond to variations in P concentrations throughout the core. While the original increase in primary producers from the Apopka-Beauclair canal construction rapidly increased primary producers in Lake Carlton, the change from diatom to cyanobacteria dominance does not seem to coincide to any direct impact upstream.

*Nutrient Inputs from Lakes Apopka and Beauclair into Lake Carlton*

According to the Mount Dora Historical Society (https://ci.mount-dora.fl.us/317/History-of-Mount-Dora), a railroad line was extended to Mount Dora in the mid-1890s. This rail line

extension spurred the construction of a network of canals—including the Carlton Cut and Apopka-Beauclair Canal—throughout the Harris Chain of Lakes, which allowed the transportation of goods, tourists, and subsequently P. The increased agricultural activities spurred a dramatic increase in nutrient loading and stimulated primary production in Lake Apopka and downstream (Schelske et al. 2005). After ~1950 the greater watershed experienced another population boom and since ~1970 there have been gradual increases in population. Increased percentages of non-volatile solids could be a result of increased development and construction of residential communities during this time.

Other nutrient data support these anthropogenic alterations as well. C:N ratios display a decreasing trend, ranging from 15-17 in historic sediments to 13-10 after connection to Lake Apopka in 1893 CE. Trends in C:N sediment records from Lake Carlton match records presented in previous work (Schelske et al. 2005, Waters et al. 2015). These decreasing C:N ratios suggest a possible change from a macrophyte-dominated system to increased algal production (Wetzel 2001, Meyers and Terranes 2001). Kenney et al. (2010) showed that many lakes within the Harris Chain of lakes experienced similar changes to C:N following the construction of the canal network. While Carlton was not in the direct string of lakes, it was connected to Beauclair after 1890 CE, and appears that similar ecological changes occurred as in the other lakes in the chain.

These changes are also made evident by the decrease in N:P ratios. Historic N:P values decrease from 25 to 10 before leveling off around ~3 after 1950 CE. Alterations in nutrient dynamics suggest changes in the source and volume of organic matter within Lake Carlton. With large increases in P loading, N:P ratios could be skewed to decrease. Also, due to its gaseous component, N burial in the sediment does not typically result in long-term storage. N deposition in sediments can be skewed by other external factors, including rates of microbial respiration that

are not typically measured in paleolimnological studies (Finlay et al. 2013). Low N:P ratios could also favor cyanobacterial production in the water column as there are many N-fixing genera of cyanobacteria (Wetzel 2001). This selection for cyanobacteria is problematic for lake managers as cyanobacteria can outcompete other phytoplankters and increase turbidity, which shades aquatic plants and macrophytes. Cyanobacterial dominance can also be concerning to lake users, as some genera of cyanobacteria are known to produce toxins hazardous to human health (Carmichael 1992).

*Primary Producer Alterations from Nutrient Inputs*

While P inputs and the initial primary producer increase appear to be linked to the Apopka-Beauclair canal, Lake Carlton experienced a significant alteration from diatom dominance to cyanobacteria dominance around ~1970 CE. The change from diatom to cyanobacteria dominance has been shown in other pigment studies on tropical and subtropical lakes (Waters et al. 2021, Waters et al. 2015). Cyanobacteria respond to disturbances better than diatoms and usually develop dominance in highly disturbed systems. Given the timeline of human impacts in the Mt. Dora area, local alterations to material inputs and landscape changes within the small Lake Carlton watershed could come from a variety of sources. Likewise, as P increased in the system, many systems within the Harris Chain of lakes reach tipping points causing regime changes to cyanobacteria dominance (Kenney et al. 2002, 2010). As higher trophic status is achieved through increased P loading, algal production and inorganic N uptake are stimulated (Schindler 2012). As these algae die, they settle to the bottom and act as a labile source of energy for heterotrophic metabolism (Tartari and Biasci 1997). This increased microbial activity decreases dissolved oxygen concentrations, which increases denitrification (Seitzinger 2006). This continual P loading (Fig 3.3) coupled with increased denitrification gives

nitrogen-fixing cyanobacteria a competitive advantage over other primary producers (Paerl 2008). Low N:P ratios (Fig 3.3) and increases in aphanizophyll concentrations (nitrogen-fixing cyanobacteria; Leavitt and Hodgson 2001) (Fig 3.4) support the inference that nitrogen limitation and cyanobacterial advantages could have led to the primary producer alterations exhibited in Lake Carlton.

*Cyanotoxin Production in Lake Carlton*

Cyanotoxins show a drastic increase in recent sediments of Lake Carlton from ~2005 to ~2015 CE. Though there are smaller increases in cyanotoxin concentration throughout the core beginning around the time of canal construction, this large increase in the upper sections of the core is an order of magnitude higher than other observed concentrations. There is a significant lag between peak cyanobacterial abundance and cyanotoxin concentration, which has been shown in other lake systems (Waters et al. 2021; Waters 2016). Echinenone is a photosynthetic pigment thought to be associated with cyanobacteria capable of producing microcystin (Leavitt and Hodgson 2001, Waters et al. 2021), and my results show a distinct relationship between microcystin and echinenone in Lake Carlton ($r^2 = 0.47$). Though the sharp drop-off in toxin concentration resembles a degradation curve, microcystins are chemically stable. Their toxicity remains after boiling (Harada, 1996) and can persist for years when stored dry and at room temperature (Metcalf et al. 2012). Rapid chemical hydrolysis is also unlikely outside of laboratory conditions (Harada, 1996). Likewise, other records covering similar time periods that measure microcystins have demonstrated similar trends with dramatic increases in the last few decades (Efting et al. 2011, Zapesta et al. 2017). When considering longer timescales, previous studies have also produced cyanotoxin records spanning thousands of years (Waters 2016; Waters et al. 2021). While sedimentary cyanotoxin interpretations are still being developed, my

measurement of total microcystins suggests that cyanotoxin production in Lake Carlton began with the onset of P inputs from the canal but reached higher levels in only the last few decades.

*Implications for Hydrologically Connected Lake Systems*

This work shows the importance of hydrologic connectivity and how it can impact nutrient dynamics and primary productivity in connected lake systems. With the construction of the Apopka-Beauclair Canal, excess nutrient pollution was able to move downstream from Lake Apopka and into Lake Beauclair and finally to Lake Carlton. These nutrient inputs immediately stimulated primary production, and coupled with local sources, caused hypereutrophic conditions in Lake Carlton resulting in a primary producer community shift. As nutrient dynamics changed over time, low N:P ratios favored cyanobacteria and triggered a replacement from diatoms to cyanobacteria as the dominant primary producers in the system. This cyanobacterial dominance gave rise to increased concentrations of cyanotoxins, which can be hazardous to lake users and lake biota. However, decreases in photosynthetic pigments and nutrient concentrations at the top of the core indicate that local nutrient pollution mitigation programs may be working. Ongoing restoration efforts in Lake Apopka and the Lake County Nutrient Reduction Facility located on the Apopka-Beauclair Canal could help mitigate the excess nutrient pollution that has negatively impacted water quality in the Harris Chain of lakes for more than 5 decades.

**Figures:**

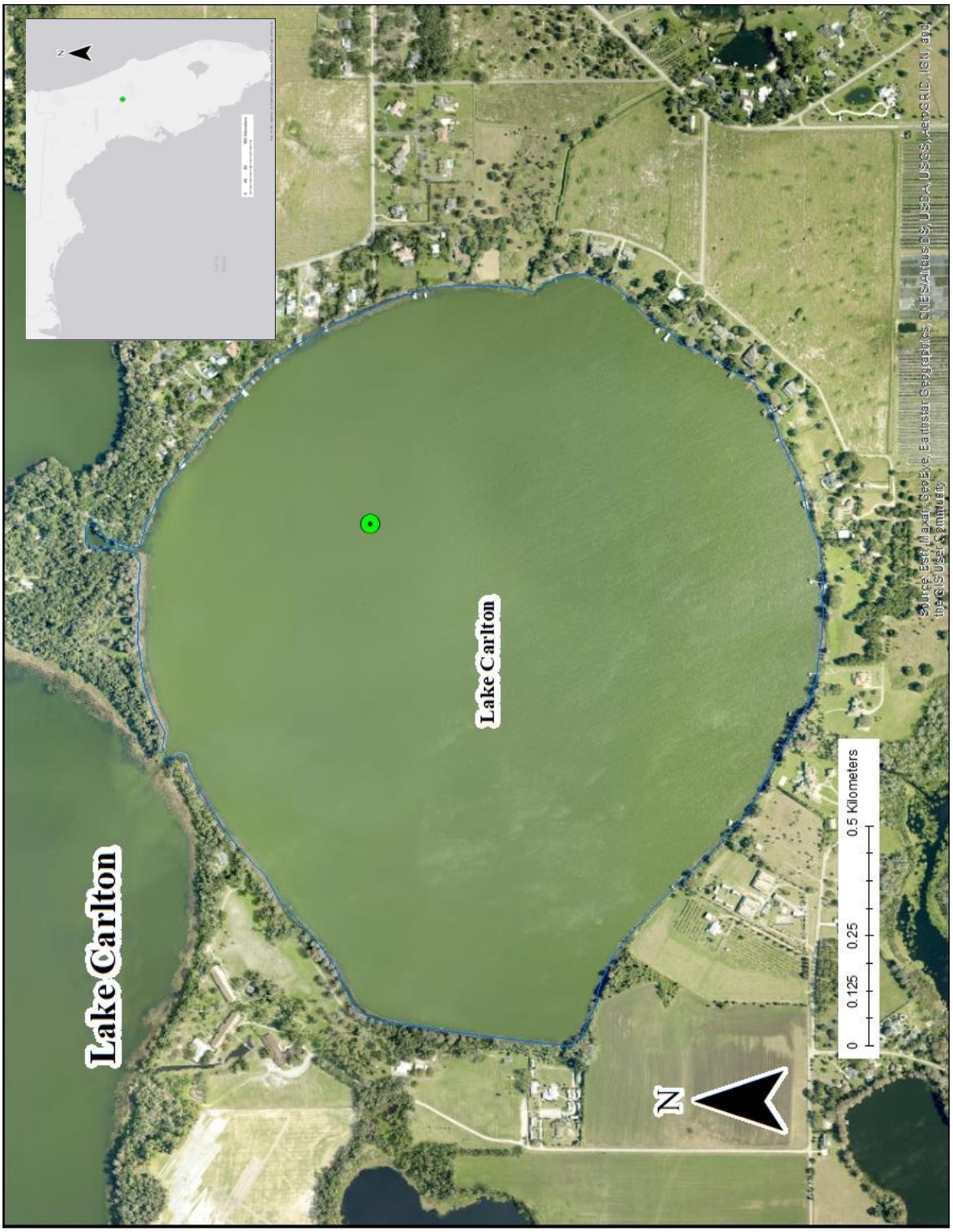


Figure 3.1: Map depicting Lake Carlton and the coring location (green dot)

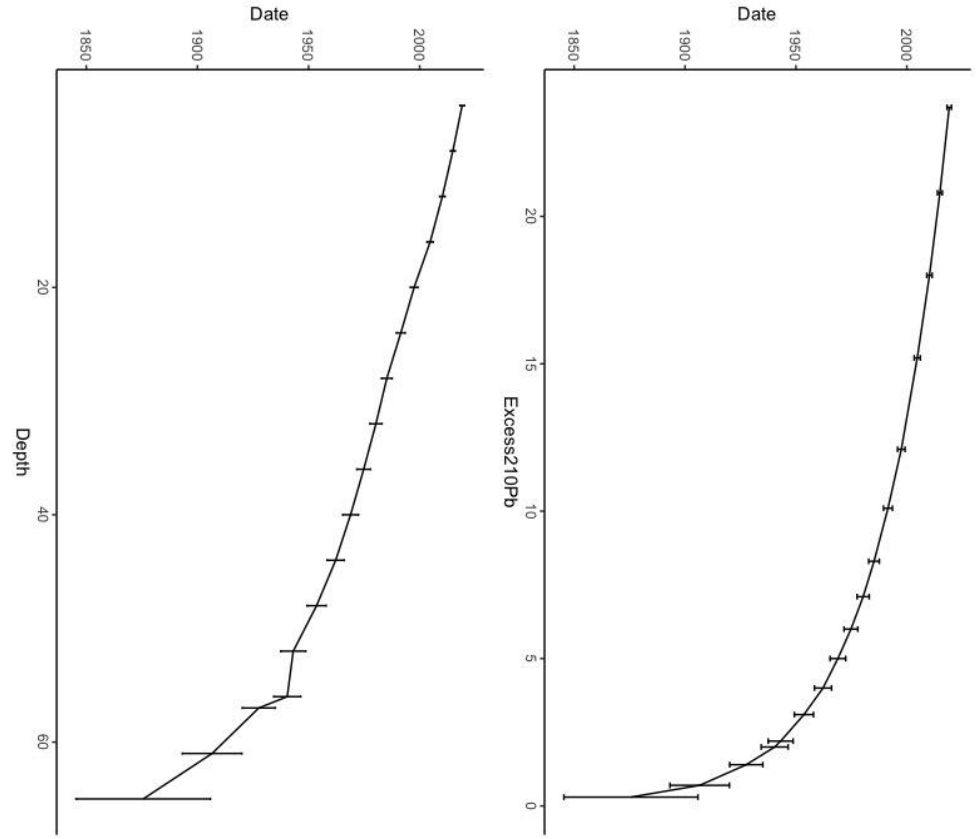


Figure 3.2: Depth and Excess $Pb^{210}$ versus depth for the Lake Carlton core. "Excess" $^{210}Pb$ activity is calculated as the difference between total $^{210}Pb$ activity and supported $^{210}Pb$ activity. Atmospheric deposition of $^{210}Pb$ occurs at a constant rate and, assuming that the lake morphology remains constant over time, can be used to assign dates to sediment samples through the Constant Rate of Supply Model (CRS) (Appleby 1983). By direct gamma counting (Appleby 1986), I was able to construct a dating model for Lake Carlton spanning the last ~150 years.

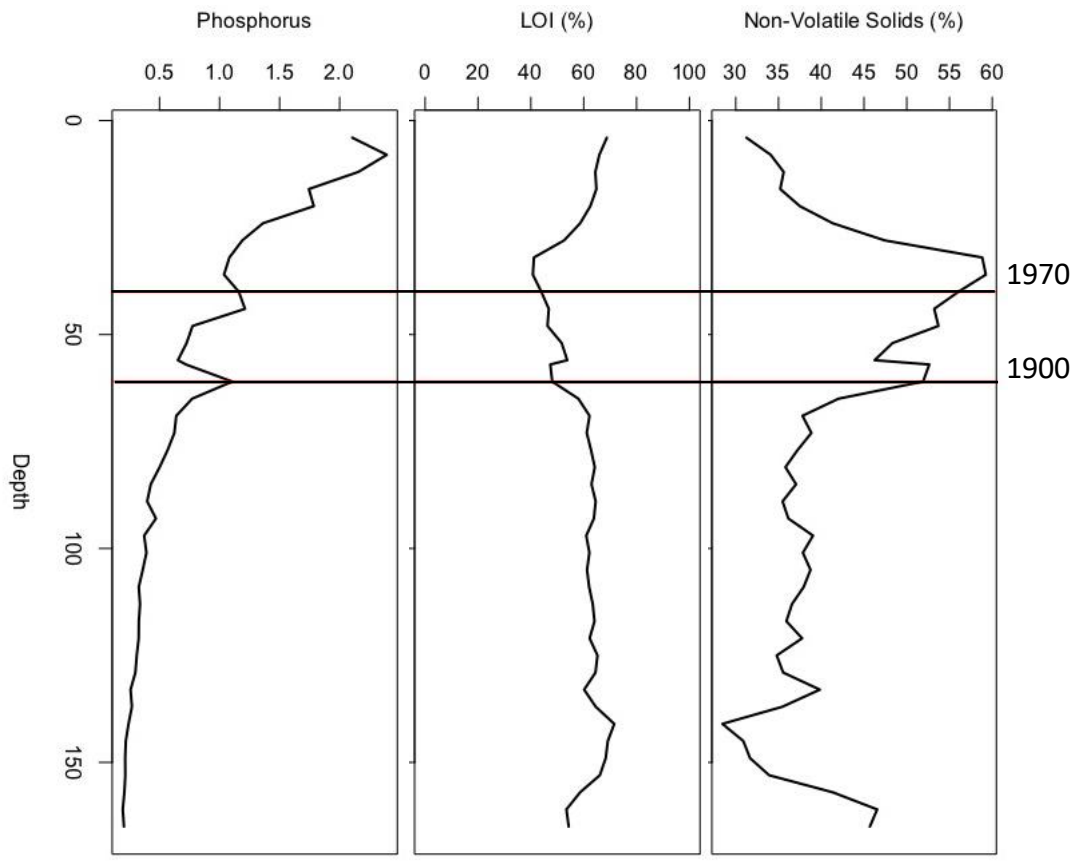


Figure 3.3: Sedimentary phosphorus concentrations, percent non-volatile solids, and organic content as loss on ignition versus depth from Lake Carlton, FL, USA. Date lines were determined from excess $^{210}$Pb.

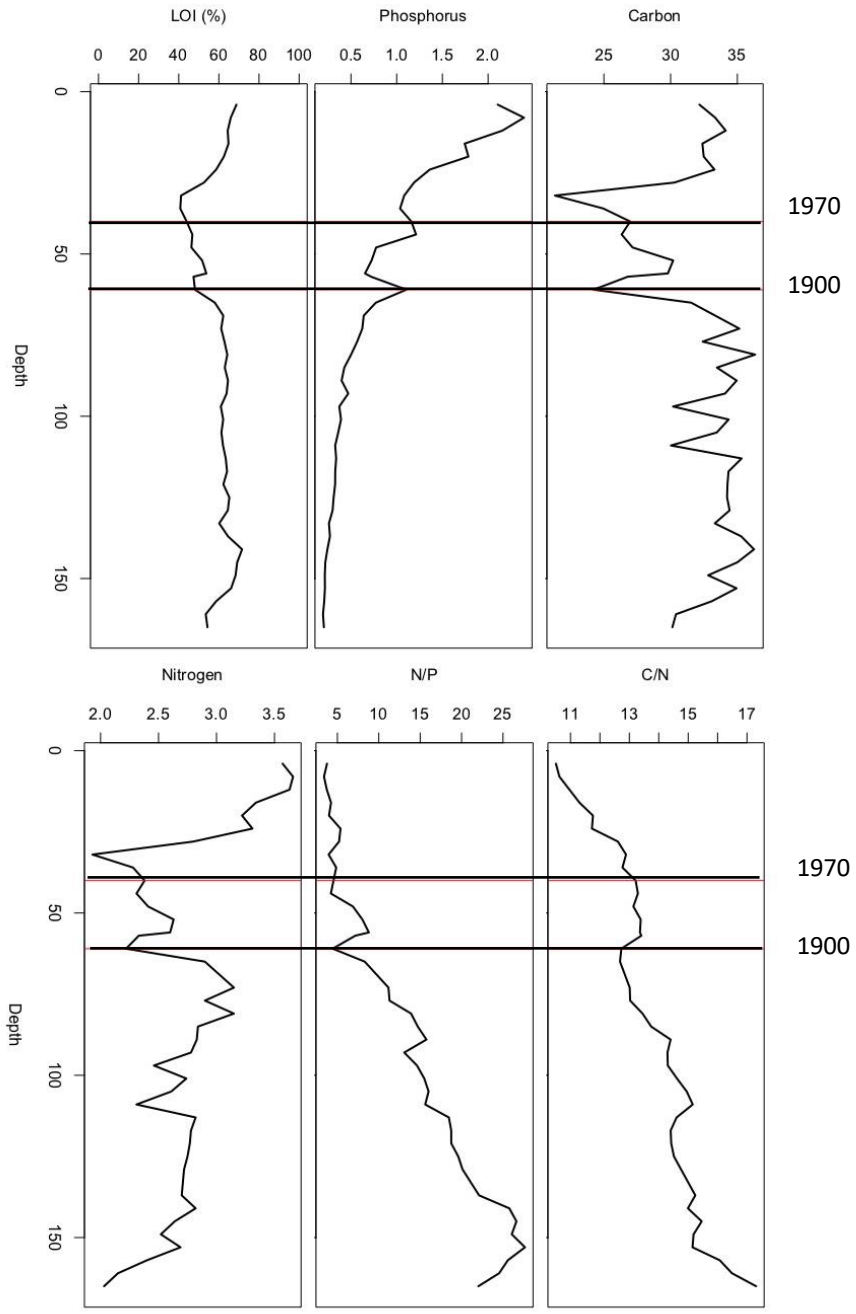


Figure 3.4: Sedimentary records for organic content (as loss on ignition), non-volatile solids, phosphorus (mg $g^{-1}$), carbon (%), nitrogen (%), carbon to nitrogen ratio, and nitrogen to phosphorus ratio versus depth for Lake Carlton. Dates are determined for excess $^{210}Pb$ measurements and the CRS model.

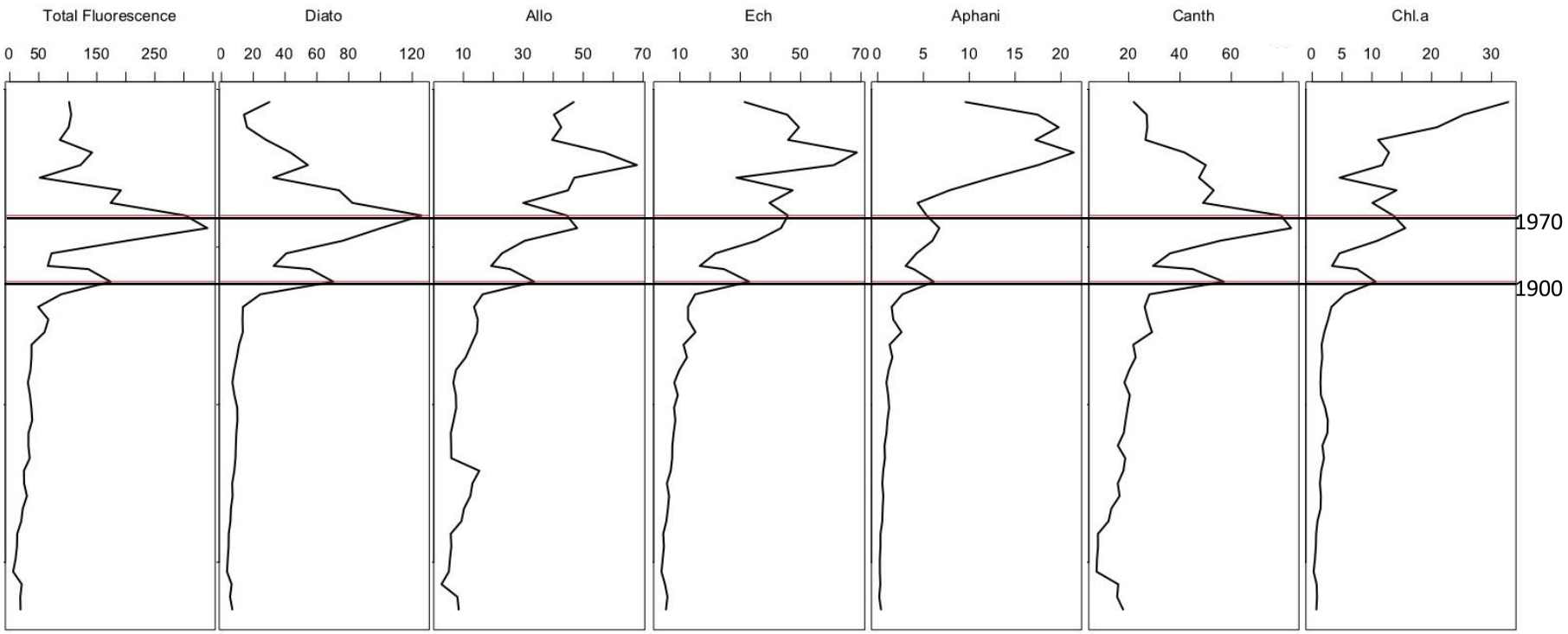


Figure 3.5: Photosynthetic pigment records versus depth from Lake Carlton: total florescence, diatoxanthin (diatoms), alloxanthin (cryptophytes), canthaxanthin (cyanobacteria), echinenone (cyanobacteria), aphanizophyll (N2-fixing cyanobacteria), and chlorophyll a (Leavitt and Hodgson 2001). Pigments are reported in nmol pigment $g^{-1}$ organic matter. Dates are determined for excess $^{210}Pb$ measurements and the CRS model.

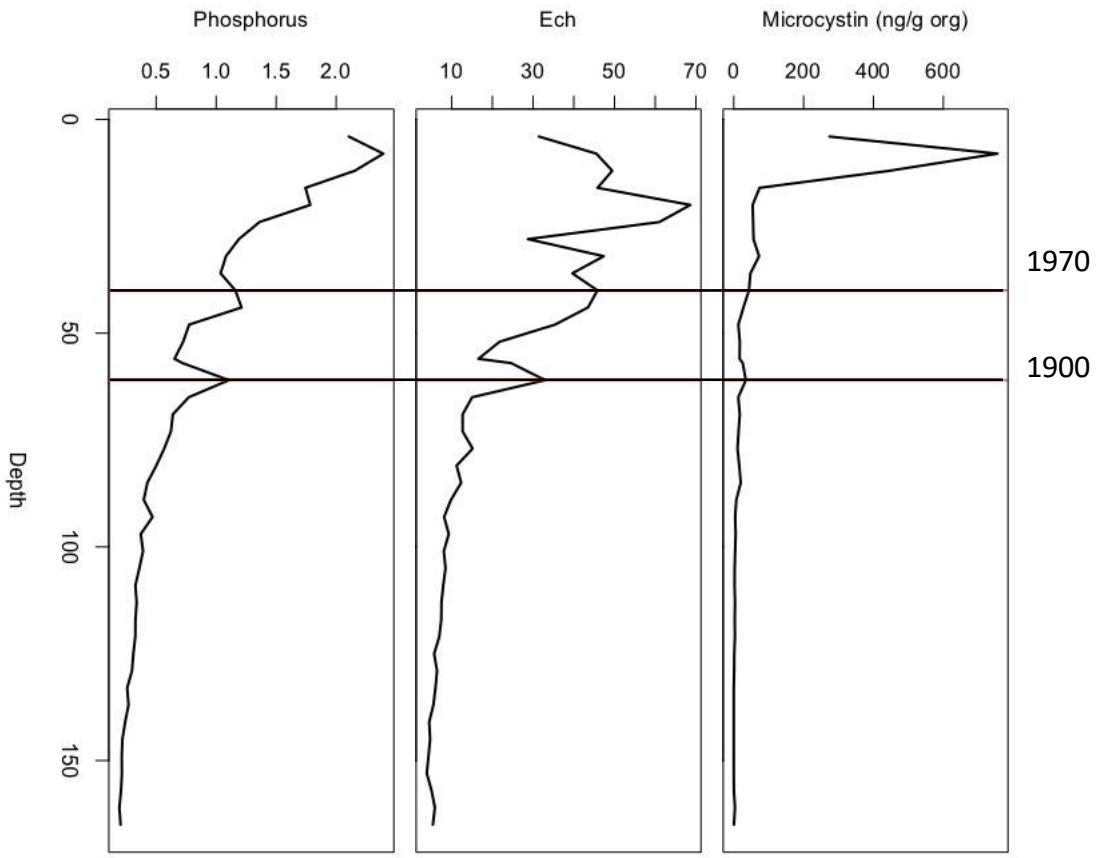


Figure 3.6: Sedimentary records of phosphorus, echinenone, and microcystin versus depth for the Lake Carlton core. Dates are determined for excess $^{210}$Pb measurements and the CRS model.

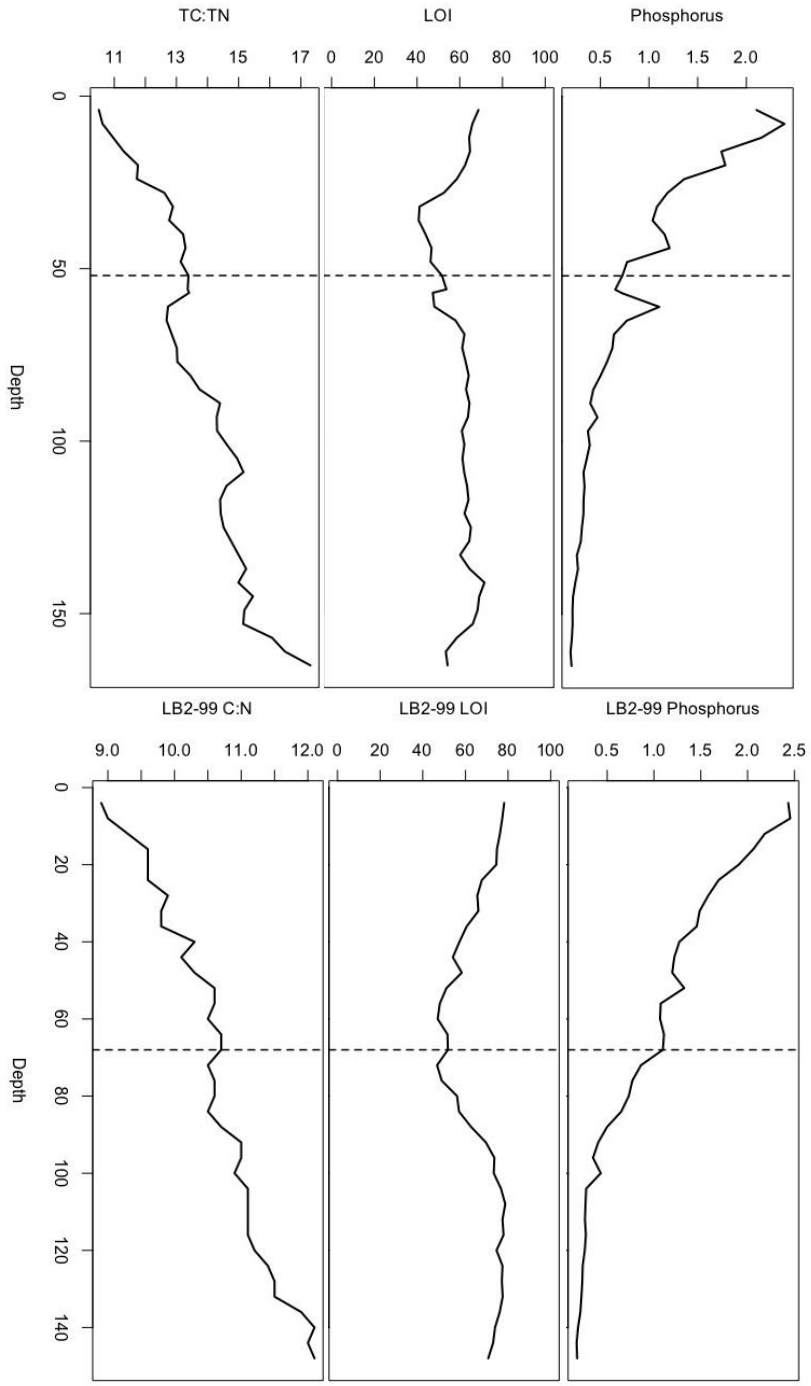


Figure 3.7: This figure shows data from this study for TC:TN ratios, TP, and NVS from Lake Carlton and TC:TN ratios, TP, and NVS data from Lake Beauclair from Schelske 2005. The dotted lines on each figure represent 1947, which is believed to be the date when Lake Apopka turned into an algal-dominated system.

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