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Characterizing ancient chemoclines through the use of pigment biomarkers and sedimentary stable isotope signatures

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Abstract:

This dissertation focuses on identifying and qualifying chemocline dynamics, namely depth and stability, in stratified aquatic systems through the use of sedimentary pigments and the stable carbon and nitrogen isotopes of organic matter. The central aim of the research comprising this volume is to identify how chemocline fluctuations are expressed in the pigment and stable isotope signatures of aquatic sediments, and how those fluctuations may have impacted nutrient cycling in past intervals of marine anoxia and mass extinction.

In order to help gauge how the depth of the chemocline may affect specific pigment signatures and concentrations in sediments, I first investigated how pigment distributions changed in microbialites over a shallow depth gradient. The immobile nature and relative stability of the environment surrounding the microbialites allowed for the investigation of how depth, and by extension changing light regime, can affect microbial community structure and the production of light harvesting vs. photoprotective pigments. It was found that the concentration of the photoprotective pigment scytonemin and its abundance relative to that of chlorophyll a decrease logarithmically with depth, consistent with the function of scytonemin as a UV screening pigment. As well, the increase in the concentration of chlorophyll a, b and the photosynthetic accessory carotenoids fucoxanthin and β-carotene with depth are consistent with lower irradiance at depth. The distribution and relative abundance of photosynthetic and light shielding pigments therefore, may provide a means for determining the relative water depth/incident radiation levels of ancient microbialites in which pigments or their derivatives are preserved. As well, it provides a modern proof of concept for utilizing changing pigment concentrations and ratios in an aquatic system to reconstruct past changes in light regime, or the depth of the locus of primary production.
The next phase of this research was then to investigate how pigment and stable isotope signatures varied in a modern stratified system during periods of known chemocline fluctuations. For this, I turned to the sediments of Lake Kivu, East Africa, a deep meromictic lake that has experienced large scale mixing events and chemocline destabilizations in the past. Within the studied core, sediments deposited coevally with mixing events exhibited distinct pigment, and carbon and nitrogen stable isotopic signatures (δ¹³C₂org and δ¹⁵Nbulk respectively) compared to surrounding background sediments. The δ¹³C₂org and δ¹⁵Nbulk values displayed sharp negative excursions at the base of the high TOC sapropel layers that are associated with the mixing events. These negative excursions provide evidence for the greater influence of ¹³C-depleted dissolved inorganic carbon and ¹⁵N-depleted ammonium derived from below the chemocline. Additionally, ratios of zeaxanthin:chlorophyllone (photoprotective and photosynthetic pigments respectively) display enormous fluctuations and spikes in the studied interval, with the sapropel layers hosting the highest values. This coupled with the presence of bacteriochlorophyll derivatives is further evidence for the breakdown of permanent stratification and shallowing of the chemocline during sapropel deposition. This study provides a mechanistic link to the strongly depleted δ¹⁵Nbulk values in the black shales of Mesozoic OAEs, and other anoxic basins of the past and can bolster predictions on the effects of future warming and deoxygenation on nutrient cycling in the modern ocean. Additionally, it provides a set of stable isotope and pigment signatures that can be used to characterize chemocline dynamics in ancient sedimentary sequences.

The final phase of this body of work characterizes chemocline dynamics in a more ancient sedimentary system, namely, the black shales from the Appalachian and Illinois basins, associated with the Late Devonian Frasnian-Famennian biotic crisis. The Frasnian-Famennian
biotic crisis marked by two distinct intervals known as the Lower and Upper Kellwasser Events that in many locations are associated with deposition of organic-rich shales. Black shales from the Illinois and Appalachian basins, including the Kellwasser Events, are $^{15}$N-depleted and have significantly lower $\delta^{15}$N$_{\text{bulk}}$ than interbedded grey shales, a trend consistent with many instances of black shale deposition in the Phanerozoic. Organic carbon isotopes exhibit the broad, positive excursions (~+3.5 ‰ from background) that are typical of the KWEs globally. Superimposed over these positive excursions in $\delta^{13}$C$_{\text{org}}$ are sharp decreases within the black shale beds. The pattern of $\delta^{15}$N$_{\text{bulk}}$ and $\delta^{13}$C$_{\text{org}}$ values suggests that, similar to Lake Kivu, the depth/stability of the chemocline and the degree of water-column stratification exert a primary control on both $\delta^{15}$N$_{\text{bulk}}$ and $\delta^{13}$C$_{\text{org}}$ during black shale deposition. In the context of the Frasnian-Famennian biotic crisis, the oscillating redox state and changing temperatures would have likely placed extreme stress on organisms within the marine environment of the Appalachian and Illinois basins, and may potentially have been a contributing factor to diversity loss over this time period.
Characterizing ancient chemoclines through the use of pigment biomarkers and sedimentary stable isotope signatures

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B.Sc., McGill University, 2013

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CHAPTER 1: AN INTRODUCTION

1.1 Introduction:

The modern ocean is losing oxygen due to warming with a significant trend observable over just the last 60 years (Breitburg et al., 2018; Schmidtko et al., 2017). This, coupled with increased nutrient runoff from anthropogenic sources has led to the spread of hypoxic ‘dead zones’ in coastal regions (Diaz and Rosenberg, 2008) and the effects will likely worsen in the coming decades (Keeling et al., 2010). The growth of regions of low oxygen waters has the potential to drastically alter the cycling of marine nutrients, specifically nitrogen and phosphorous, as these elements are processed and cycled differently under such conditions (Benitez-Nelson, 2000; Galbraith and Sigman, 2008; Higgins et al., 2012; Junium and Arthur, 2007; Mort et al., 2007; Van Cappellen and Ingall, 1994). Because the modern ocean is currently still well oxygenated overall, we must look to analogue modern systems and to instances of widespread ocean anoxia in the past in order to better constrain the effects of future warming on marine ecology and nutrient cycling (Jenkyns, 2010).

The geologic record of the Phanerozoic is punctuated with numerous intervals of widespread deoxygenation and anoxia, the most well-known being the Ocean Anoxic Events (OAEs) of the Mesozoic (Jenkyns, 2010; Karakitsios et al., 2010; Schlanger et al., 1987; Schlanger and Jenkyns, 1976). OAEs represent enormous climatic, chemical, and ecological changes in in the ocean, and are typically associated with massive increases in greenhouse gases and warming (Barclay et al., 2010; Jenkyns, 2010; Leckie et al., 2002; Schlanger and Jenkyns, 1976). Even events not counted amongst the ‘classic’ OAEs, such as the Frasnian-Famennian Biotic Crisis (Bambach, 2006; Racki, 2005), the Permo-Triassic Mass Extinction (Erwin, 1994;
inflicted far-reaching effects on marine ecology (Bambach, 2006; Caruthers et al., 2013; Erwin, 1994; Harries, 1993; Harries and Little, 1999; Jablonski, 1991; Racki, 2005; Speijer et al., 2012; Wignall and Hallam, 1992) and nutrient cycling (Junium et al., 2018; Mettam et al., 2017; Uveges et al., 2018). These events are widely thought to be associated with a strengthened degree of water column stratification and the presence of a chemocline separating the oxygenated upper water column from the dysoxic-anoxic and often sulfidic lower water column.

An active area of active scientific interest lies in characterizing the stability and depth of the chemocline as well as the areal distribution of anoxic/euxinic deep waters through the use of biomarkers (Brocks et al., 2005; Gloe et al., 1975; Keely, 2006; Koopmans et al., 1996), basin scale sedimentology (Ettensohn and Elam, 1985), and trace metal geochemistry (Algeo et al., 2007; Algeo and Rowe, 2012; Little et al., 2015; Song et al., 2017; Tribovillard et al., 2006). The relevance of these studies is rooted in the ability of anoxic deep waters to regenerate and trap nutrients, particularly phosphate and ammonium which can fuel enhanced primary production, compounding anoxia (Higgins et al., 2012; Van Cappellen and Ingall, 1994) and its effects on marine ecology, as well as the potential positive feedbacks associated with chemical cycling and greenhouse gases that can enhance warming (Canfield et al., 2010; Voss et al., 2013).

An additional outstanding issue in the study of OAEs and other deoxygenation events is the presence of anomalously low/negative nitrogen isotope values in the coeval sediments. Nitrogen isotopes in sedimentary systems generally lie above the diazotrophic nitrogen fixation input end member of -2 – 0‰ (Bauersachs et al., 2009; Wada, 1980). There is some evidence for greater fractionation associated with the use of alternative nitrogenase enzymes for fixation (Pau
et al., 1993; Zhang et al., 2014), however, these enzymes have not been observed to be in use outside of a few isolated terrestrial and coastal environments (Betancourt et al., 2008; Darnajoux et al., 2016). A alternative proposed mechanism for these depleted sedimentary values is the increased utilization of ammonium derived from below a shallow chemocline either by organisms living at/below the chemocline interface, or by surface producers during mixing events (Higgins et al., 2012; Junium et al., 2018; Junium and Arthur, 2007) however, there are few modern environments in which we can test this hypothesis, or past studies with high enough sampling resolution to capture short term fluctuations. This body of research aims to characterize past environments and chemocline dynamics through the use of pigment biomarkers, and the stable isotopes of carbon and nitrogen, in order to help better constrain and predict the effects of modern/future warming on marine nutrient cycling.

1.2 Research questions:

I.) How are pigment distributions affected by depth in a water column in living organisms from a modern system?

II.) Can we utilize sedimentary pigments and C and N isotopic signatures to characterize inferred intervals of chemocline variations/instability?

III.) Do chemocline variations/destabilizations provide a feasible mechanism for negative nitrogen and carbon isotope excursions during past instances of deoxygenation, such as the Late Devonian?
1.3 Methods and conceptual framework:

1.3.1 Instrumentation and methodology:

1.3.1.1 Stable isotope mass spectrometry:

The advent of the mass spectrometer (Rutherford, 1905) has allowed for the discrimination of both radioactive and stable isotopes of elements, and for the measurement of the relative abundances of these isotopes since the first isotope ratio measurement of xenon (Thomson, 1913). The first idea that natural processes could fractionate light isotopes arose in 1925 based on measurements of boron (Briscoe and Robinson, 1925) and evolved into the first effective application to a geochemical problem with an attempt to calculate the total amounts of coal and bitumen in the Earth (Wickman, 1941). Subsequently, geochemical and paleoclimatic applications began to rapidly expand after the ground breaking discovery that paleotemperatures could be measured through the oxygen isotopes of carbonates (Epstein et al., 1951; Urey, 1948; Urey et al., 1951). Since then, stable isotope ratios have been shown to be faithful recorders of a panoply of geochemical and climatic processes (Sharp, 2007).

The basic method behind the stable isotope mass spectrometry utilized in this dissertation is relatively straightforward. First, the investigated samples must be converted into a gas, generally through combustion; second, the element of interest (in this case carbon in the form of CO\textsubscript{2} and nitrogen in the form of N\textsubscript{2}) must be separated from other gases evolved from the sample; third, the gas must be ionized so that it can be influenced by a magnetic field; fourth, the ionized gas must then be passed through a magnetic field of a particular strength in order to separate the gas into distinct beams of a single mass; finally, the ions beams must be passed through a detector, where they generate a measurable electric current that can be translated to relative abundances of each isotope. The Isotope studies described herein were performed in the Syracuse University
GAPP Lab using an Elementar Isotope Cube elemental analyzer (EA) coupled directly to an Isoprime 100 isotope ratio mass spectrometer (IRMS) using conventional techniques for EA-IRMS. Solid crushed sediment samples were first acidified using 3 N hydrochloric acid to remove carbonate phases, dried, and weighed into tin cups. Samples were then introduced to the EA system, where they were oxidized in an 1100°C oxidation reactor comprised of aluminum oxide wool/spheres (Al₂O₃) and tungsten (VI) oxide (WO₃). The evolved gas is taken up in a helium stream and passed to a 650°C reduction furnace comprised of reduced copper (Cu) filings, quartz (SiO₂) wool and silver (Ag) wool where oxidized nitrogen species (NOₓ) are reduced to N₂.

![Figure 1-1: Elementar Isotope Cube elemental analyzer (EA) coupled directly to an Isoprime 100 isotope ratio mass spectrometer (IRMS)](image)

The reduced gases were then directed through water and CO₂ traps, and a thermal conductivity detector (TCD) in order to quantify the amount of a given element in the sample (Fig. 1). When measuring carbon isotopes, the trapped CO₂ was released to the sample stream once other gasses
were allowed to pass through. The sample stream was then introduced to the magnetic sector IRMS through a sample diluter, ionized via electrons produced by a tungsten-thorium filament, and sent through a magnetic field/flight tube where the individual mass species are separated based on the Lorentz law (see Ireland, 2013 for overview). The ions then reach an array of Faraday cup collectors where they generate a measurable current that is used to derive the ratio of the heavy to the light isotope ($^{13}\text{C}/^{12}\text{C}$ for carbon, $^{15}\text{N}/^{14}\text{N}$ for nitrogen). Isotope ratio values are then converted to delta ($\delta$) notation, which is the comparison of the sample ratio, to the ratio of a universally accepted standard (see equation 1 and 2)(Coplen et al., 2006, 2002). More detailed instrument settings, conditions, and standard details can be found within chapters 3 and 4.

\[
(1) \quad R = \frac{\text{amount of } ^{13}\text{C}}{\text{amount of } ^{12}\text{C}}
\]

\[
(2) \quad \delta^{13}\text{C} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000\%
\]

1.3.1.2 HPLC separation and UV-Vis analysis of pigments

High performance liquid chromatography (HPLC) has been shown to have broad utility in the separation of complex mixtures of organic compounds, particularly those which contain compounds that are non-volatile or strongly polar and are not amenable to separation by gas chromatography (GC) (Airs et al., 2001). The key features of an HPLC system are 1.) A pump used to force solvents (mobile phase) through the system; 2.) A sample injection mechanism which introduces the sample into the solvent stream; 3.) A column filled with stationary phase having a different polarity than that of the mobile phase solvents, that promotes separation of the
individual components of the mixture based on their polarity/structural flexibility; 4.) A detector capable of quantifying the eluted compounds (Fig. 2).

In this body of research, mixtures of pigments were separated and analyzed using a Thermoscientific Dionex Ultimate 3000 UHPLC system equipped with a quaternary solvent pump loaded with four solvents; water, acetonitrile, methanol and ethyl acetate (listed in order of decreasing polarity). Samples were introduced using a 50 µl injection loop auto sampler, and then passed through two Waters Spherisorb reverse phase (nonpolar) analytical C18 silica columns linked in series (Fig. 2). Separation was achieved utilizing a gradient elution scheme where the proportion of the solvents applied to the column changed over the course of the analysis, moving from more polar to less polar, following method A of Airs et al. (2001). The onboard detector in this system is a UV/Visible light photodiode array which quantifies pigments based on their absorbance of a given wavelength(s). Light is generated by deuterium (UV) and tungsten (Vis) lamps, and then passed through a quartz cuvette hosting the solvent stream, and then on to a diffraction grating which reflects the individual wavelengths of light at different angles. The separated light then passes to a photo diode array where a current is generated based on the intensity of the light reaching each diode. As a sample compound is passed through the quartz cuvette, it will absorb specific wavelengths of light, and the current being generated in those specific photodiodes will drop and this drop in current is proportional to the concentration of the analyte passing through the cuvette according to Beers Law (equation 3), where A is absorbance (in arbitrary units AU), e is the molar absorption coefficient, L is the path length of the light through the cuvette, and C is the concentration of the analyte (see review by Skoog et al., 2007).

\[
(3) \quad A = eLC
\]
1.3.1.3 LC-MS\textsuperscript{n} analyses of pigments

After passing through the diode array detector, separated compounds proceed to the liquid chromatography ion trap multistage mass spectrometer (LC-MS\textsuperscript{n}). Similar to stable isotope mass spectrometry, the analytes are first volatilized and ionized. Volatilization is achieved using a heated nebulizer, and ionization through atmospheric pressure chemical ionization (APCI). APCI involves passing the nebulized solvent and sample mist across a corona discharge electrode needle along with the nebulizing gas (Hoffman and Stroobant, 2001) (Fig. 3). A primary ion is then formed by ionizing the nebulizing gas (in this case N\textsubscript{2} \rightarrow N\textsubscript{2}\textsuperscript{+}), which then collides with solvent molecules to create the secondary ion (in this solvent system H\textsubscript{2}O \rightarrow...
H$_3$O$^+$). From the secondary ion, a cascade of chemical reactions proceeds until the analyte (M) is ionized through proton transfer (MH$^+$) or adduct formation (e.g. MNa$^+$) (Fig. 3).

![Diagram](image)

**Figure 1-3:** Schematic of Thermo LCQ Fleet Ion Trap LC-MS$^n$.

The ionized analyte then proceeds through a skimmer/focusing cone into the quadrupole ion trap mass spectrometer, which is made up of four electrodes: two ring electrodes and two end cap electrodes (Fig. 3). Sample ions are introduced to spectrometer through an aperture in the entry end-cap electrode where an oscillating radio frequency (RF) potential, that is applied across the ring electrodes, effectively “traps” the ions in the center of the electrodes. In order to mitigate the expansive forces brought on by like-charged ions in close proximity, a cooling helium buffer
gas is applied at a constant pressure. The instrument then undergoes a “mass scan” where the amplitude of the RF potential is gradually increased. As this potential increases, ions of increasing mass/charge (m/z) ratio can effectively escape through the exit end cap electrode and to the detector. Ions of a particular (m/z) ratio can be isolated within the trap through resonance ejection of all other ions utilizing a supplemental AC potential that matches with a specific ions’ secular frequency (a frequency at which an ion with a given m/z oscillates within the trap). Once isolated, trapped precursor ions are fragmented via resonance enhanced collision induced dissociation (CID) through collision with the helium cooling gas creating fragment MS² ions. The MS² ions can then either be passed on to the detector, or isolated and further fragmented into MS³ ions and so on. This process can be repeated \( n \) times until the number of MS\(^n\) ions remaining falls below the detection limit. This process provides a wealth of information on the structural properties and functional groups of a compound of interest (Rivera et al., 2014; Rosell-Melé, 1999). For the studies detailed in chapters two and three, the depth of the mass fragmentation was limited to MS⁴ due to the complexity of the mixtures being analyzed.

1.3.2 Brief overview of proxies used

1.3.2.1 Nitrogen isotopes

Nitrogen isotopes (\( \delta^{15}\text{N} \)) serve as a proxy for nutrient dynamics, sources of biologically available nitrogen, and the degree to which redox processes affect the isotopic composition of reactive nitrogen reservoirs. Sedimentary organic matter \( \delta^{15}\text{N}_{\text{bulk}} \) values have been shown to be reliable tracers of sub-euphotic zone nitrate, and therefore can be used to track the evolution of nitrogen cycling processes at a particular site through time (Robinson et al., 2012; Tesdal et al., 2013; Thunell et al., 2004). The \( \delta^{15}\text{N} \) of nitrate is controlled by the balance of processes that act
to add or removed nitrogen from the ocean (Algeo et al., 2014; Altabet, 2007; Brandes and Devol, 2002; Deutsch et al., 2004), which are largely regulated by water column redox state and N:P ratios (Loladze and Elser, 2011; Quan and Falkowski, 2009).

**Figure 1-4:** Overview of the major processes in the marine nitrogen cycle

Most new dissolved inorganic nitrogen (DIN) is introduced to a marine system by diazotrophic nitrogen fixation, which is an energy intensive process due to the need to reduce triple-bound diatomic nitrogen gas across three oxidation states to ammonium, which also likely contributes to its low fractionation effect of -3 - +1 ‰ (Bauersachs et al., 2009; Fogel and Cifuentes, 1993; Higgins et al., 2012). In the modern ocean, fixed reduced nitrogen is generally oxidized to nitrite, and subsequently nitrate by the process of nitrification which can have a significant isotope effects (Casciotti et al., 2010) but these are generally not expressed due to
quantitative utilization. Nitrate reduction (denitrification), and anaerobic ammonium oxidation (anammox), which occur in anoxic water columns and sediments (Brandes et al., 2007; Canfield et al., 2010), are the principal sinks for DIN in the ocean (e.g. Lam et al., 2009; Ward et al., 2009) (see Fig. 4 for overview of marine nitrogen cycle). Rates of diazotrophy and denitrification/anammox show a degree of spatial coupling as the loss of DIN acts to decrease the N:P ratio in the water column, creating ecologically advantageous conditions for phosphorus dependent diazotrophs once DIN has been depleted (Deutsch et al., 2007; Moutin et al., 2008). Each of these processes have an associated isotope effect, that generally act to enrich the residual nitrate pool: water-column denitrification ($\epsilon \sim +20 \%$), sedimentary denitrification ($\epsilon \sim 0 \%$, due to quantitative utilization), and anammox ($\epsilon \sim +23.5 - +29.1 \%$) (Altabet and Francois, 1994; Brunner et al., 2013; Canfield et al., 2010; Galbraith and Sigman, 2008; Hoch et al., 1994, 1992; Lam et al., 2009; Waser et al., 1998).

### 1.3.2.2 Carbon isotopes

Carbon isotopes of marine organic matter ($\delta^{13}C_{\text{org}}$) have been shown to serve as a tracer for the global carbon cycle (Kump and Arthur, 1999). However, on local scales the carbon isotopic composition of sedimentary organic matter is dependent on other factors, such as $p$CO$_2$ (Freeman and Hayes, 1992; Hollander and McKenzie, 1991), organic matter source (Meyers, 1994), microbial ecology/the biosynthetic pathway of carbon fixation (House et al., 2003; Hügler and Sievert, 2011), the $\delta^{13}C$ of dissolved inorganic carbon being utilized for organic carbon production (Fogel and Cifuentes, 1993), and the extent of heterotrophy. Because of this small-scale heterogeneity, the $\delta^{13}C_{\text{org}}$ of sediments can therefore be used to characterize localized carbon cycle dynamics through time. Used in tandem, $\delta^{13}C_{\text{org}}$ and $\delta^{15}N_{\text{bulk}}$ data can help to
illuminate the interplay of regional water-column redox structure and primary producer communities.

1.3.2.3 Pigments

Pigments are organic compounds that interact with and absorb specific wavelengths of light, dependent on their structure and electronic properties (see Fig. 5 for example structures). In microbial organisms, pigments are directly associated with photosynthesis (e.g. chlorophyll a) or serve a variety of roles in support of photosynthesis, including photoprotective roles such as UV screening. There are several forms of chlorophyll, each of which is typically produced by specific classes of photoautotrophs (Castañeda and Schouten, 2011; Loughlin et al., 2013; Roy et al., 2011). For example, notable differences in the structures of many bacteriochlorophylls allows for positive identification of anoxygenic photoautotrophs from ancient systems (e.g. Keely, 2006). Carotenoids have a broad structural and functional diversity (Rivera et al., 2014; Roy et al., 2011; Takaichi, 2011) that play important roles in light harnessing (Alberte et al., 1981; Guglielmi et al., 2005; Katoh et al., 1989; Tanada, 1951) as well as photoprotection (Ehling-Schulz et al., 1997; Garcia-Pichel et al., 1992). Carotenoids also have utility as biomarkers in both modern and ancient systems, as many carotenoids are specific to certain classes of organisms (Castañeda and Schouten, 2011; Roy et al., 2011; Takaichi, 2011). Additionally there have been numerous studies that have shown the effects of light on pigment concentrations within cultured and wild organisms (Ehling-Schulz et al., 1997; Garcia-Pichel and Castenholz, 1991; Kana et al., 1988; Kao et al., 2012; Leisner et al., 1994; Schäfer et al., 2006). As such, pigment quantification can be used to investigate contributions of specific organisms to bulk biomass, as well as assess light regimes.
Figure 1-5: Select molecular structures of common pigments. A: β-Carotene; B: Lutein; C: Fucoxanthin; D: Diadinoxanthin; E: Chlorophyll d; F: Chlorophyll b; G: Chlorophyll a; H: Hydroxy-pheophytin a; I: Pheophytin a; J: Pyropheophytin a; K: Scytonemin.
1.4 Dissertation Outline

This dissertation proceeds stepwise from a modern microbialite system to progressively more ancient sedimentary systems. In chapter 2, an analysis of the pigment distributions within modern microbialites, and how they vary with depth is presented in order to investigate how water depth can be qualified by relative pigment concentrations. It was found that the UV screening pigment scytonemin, along with chlorophyll a and other pigments displayed statistically significant correlations with depth in the water column, and UV A irradiance. This chapter has recently been accepted for publication at the journal Organic Geochemistry with coauthors Mark A. Teece, James M. Fulton and Christopher K. Junium.

Chapter 3 describes how sedimentary pigments and the stable isotope signatures of carbon and nitrogen changed in association with hypothesized chemocline destabilization events in a modern meromictic lake in East Africa (Lake Kivu) within the past several kyr. At the base of the organic-rich sapropels initiated by the volcanism/hyperpycnal flow driven mixing events, sharp decreases in δ\(^{13}\)C\(_{\text{org}}\) and δ\(^{15}\)N\(_{\text{bulk}}\) values occur, accompanied by a general increase in the ratio of photoprotective to light gathering pigments. As sapropelic deposition ends, carbon isotopes recover to background values, while nitrogen isotopes overshoot to strongly enriched values and then return to background. The patterns observed are most consistent with a delivery of high concentrations of isotopically depleted dissolved inorganic carbon and ammonium to the photic zone during mixing events, followed by a subsequent quantitative utilization of ammonium as stratification is reestablished. These mixing events likely moved the chemocline as well as the locus of primary production closer to the surface, resulting in the increased production of photoprotective pigments. This chapter is in preparation to be submitted with coauthors Christopher A. Scholz, James Fulton and Christopher K. Junium.
Finally, in chapter 4, high-resolution stable isotope analyses of carbon and nitrogen are applied to the Late Devonian Appalachian (AB) and Illinois Basins (IB) with a focus on the Kellwasser intervals associated with the Frasnian-Famennian biotic crisis. In the studied sections the isotope signatures observed were strikingly similar to those observed in Lake Kivu sediments. Black shales from the IB and AB are $^{15}\text{N}$-depleted and have significantly lower $\delta^{15}\text{N}_{\text{bulk}}$ than interbedded grey shales, and also display sharp decreases in $\delta^{13}\text{C}_{\text{org}}$ superimposed on the longer term global positive excursion. The pattern of depletion suggests that the depth of the chemocline and the degree of water-column stratification exert a primary control on both $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ during black shale deposition. In the context of the Frasnian-Famennian biotic crisis, the oscillating redox state and changing temperatures would have likely placed extreme stress on organisms within the marine environment of the AB and IB and may potentially have been a contributing factor to diversity loss over this time period. Chapter 4 has been published in the journal *Palaeogeography, Palaeoclimatology, Palaeoecology* with coauthors Christopher K. Junium, Diana L. Boyer, Phoebe A. Cohen and James E. Day.

Overall this body of work lends new insight into the influence of chemocline dynamics and the presence of ammonium on past instances of marine anoxia and nutrient cycling, with chapter 3 providing a particularly compelling analogue for the influence of chemocline variations on past sedimentary systems.
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Abstract

We analyzed pigments in acetone extracts of microbialites collected over a shallow depth gradient from the freshwater Fayetteville Green Lake (FGL), Fayetteville, NY. Pigment identification and quantification were achieved using reverse phase high performance liquid chromatography and ion trap multi-stage mass spectrometry (MS^n). Chlorophyll a and its derivatives, the photoprotective pigment scytonemin, and the carotenoid fucoxanthin were present in all samples, β-carotene was observed in all but one sample, and minor abundances of other pigments such as chlorophyll d and lutein were observed in select samples. The concentration of scytonemin and its abundance relative to that of chlorophyll a decrease logarithmically with depth, consistent with the function of scytonemin as a UV screening pigment. As well, the increase in the concentration of chlorophyll a, b and the photosynthetic accessory carotenoids fucoxanthin and β-carotene with depth are consistent with lower irradiance at depth. The distribution and relative abundance of photosynthetic and light shielding pigments may provide a means for determining the relative water depth/incident radiation levels of ancient microbialites in which pigments or their derivatives are preserved.
2.1 Introduction

Microbialites have been an important constituent of Earth’s geologic record for nearly 3.5 billion years (Grotzinger and Knoll, 1999; Hofmann et al., 1999), and are among the most common organo-sedimentary structures through the bulk of Earth’s history (Burne and Moore, 1987; Riding, 2011). The formation of microbialites is fostered by the metabolic activity of diverse microbial mat communities that catalyze the precipitation of calcium carbonate, producing laminated carbonate accumulations associated with organic matter, cellular exudates, and trapped sedimentary grains (Burne and Moore, 1987; Dupraz and Visscher, 2005; Dupraz et al., 2009; Riding, 2011). The metabolically active region of microbialite systems comprises the outer-most, mm-scale layer that includes photoautotrophs on the surface and diverse heterotrophic and chemoautotrophic organisms on the interior (Allen et al., 2010; Brady et al., 2014). The phototrophic community is commonly dominated by cyanobacteria, but also includes green algae and diatoms (Chan et al., 2014), and heterotrophic bacteria on the microbialite interior respire organic matter produced by the phototrophs (Allen et al., 2010; Brady et al., 2014).

Organic geochemical analyses of modern microbialites provide context from which we can better understand past environments. Ancient microbialites typically do not preserve the type of detailed genetic data that allow us to investigate microbial communities and the diversity of metabolisms that exist in modern microbialites (e.g. Edgcomb et al., 2013). However, lipid and pigment biomarkers have a comparatively higher potential for preservation and can provide data about the makeup of ancient communities where organic biomarkers are preserved. Investigating modern systems and the range of factors that influence pigment and lipid biomarker moieties provides this necessary context.
Pigments are organic compounds that interact with and absorb specific wavelengths of light, dependent on their structure and electronic properties. In microbial organisms, pigments are directly associated with photosynthesis (e.g. chlorophyll \(a\)) or serve a variety of roles in support of photosynthesis, including photoprotective roles such as UV screening. There are several forms of chlorophyll, each of which is typically produced by specific classes of photoautotrophs (Castañeda and Schouten, 2011; Roy et al., 2011; Loughlin et al., 2013). For example, notable differences in the structures of many bacteriochlorophylls allows for positive identification of anoxygenic photoautotrophs from ancient systems (e.g. Keely, 2006). Carotenoids have a broad structural and functional diversity (Roy et al., 2011; Takaichi, 2011; Rivera et al., 2014) that play important roles in light harnessing (Tanada, 1951; Alberte et al., 1981; Katoh et al., 1989; Guglielmi et al., 2005) as well as photoprotection (Garcia-Pichel et al., 1992; Ehling-Schulz et al., 1997). Carotenoids can also be used as biomarkers in both modern and ancient systems, as many carotenoids are specific to certain classes of organisms (Castañeda and Schouten, 2011; Roy et al., 2011; Takaichi, 2011).

Here, we present pigment biomarker analyses from modern microbialites collected from the meromictic Fayetteville Green Lake, New York, a well-studied analog for ancient oceans (Meyer et al., 2011; Havig et al., 2015, 2017; Fulton et al., 2018) that has actively accumulating thrombolitic microbialites in shallow waters (Thompson et al., 1990). The objectives of this study were to: 1) determine the pigment composition of the freshwater microbialites at FGL and 2) determine if there is variation in the type or amount of pigments relative to water column depth position of the microbialites. Characterizing the distribution and controls of pigment signatures in modern microbialites is a valuable exercise, as it can provide necessary context for
characterization of ancient microbialites and environmental conditions on Earth, as well as on other planets such as Mars (Dupraz and Visscher, 2005; Varnali et al., 2009).

Figure 2-1: Map of study area with microbialite sample locations labeled.

2.2 Geologic Setting

Fayetteville Green Lake (FGL) is a meromictic freshwater lake located near Syracuse, NY, the first lake classified as such in North America (Eggleton, 1931). The origin of the lake is assumed to be a glacial plunge pool that likely formed because of extensive erosion associated with glacial melt runoff at the end of the Pleistocene glaciation period. The lake drains an area of 4.3 km², and the predominant inflows are surface water runoff, a stream that flows into FGL from nearby Round lake and subsurface inflows of groundwater (Brunskill and Ludlam, 1969). The groundwater contains high concentrations of calcium and sulfate sourced from Silurian Age gypsum-rich Vernon Shale that subcrops beneath the lake surface (Brunskill and Ludlam, 1969). The slightly saline groundwater that enters the lake at depth contributes to the persistence of meromixis which results in a sharp chemocline that is generally between 17 - 20 m water depth
(Havig et al., 2015, 2017), and varies slightly with seasonal differences in the lake’s water balance (e.g. Fulton et al., 2018).

The entire water column is supersaturated with respect to calcite (CaCO$_3$) which lends to the precipitation of calcite associated with *Synechococcus* in the water column and active microbialite growth on the shallow margins of FGL (Thompson et al., 1990, 1997). Microbialites are found at the surface and to depths of approximately 13 meters (Wilhelm and Hewson, 2012), aided in part by the unusual clarity of the water (Brunskill and Ludlam, 1969). Metagenomic (16sRNA), microscopy and a biomarker analysis has confirmed that dominant microbial communities in FGL microbialites are cyanobacteria, with a significant proportion of diatoms incorporated into cyanobacterial biofilms (Thompson et al., 1990; Wilhelm and Hewson, 2012; Shields, 2017).

### 2.3 Experimental Information

#### 2.3.1 Sampling

The top 1cm of living microbialites were collected with hammer and chisel under New York State Parks Permit #2014-GR-005 from Green Lake in Green Lakes State Park in Fayetteville NY (Fig. 1) (September 2014). Several samples were initially covered in a thin layer of charophyte algae, which was removed prior to collection. Samples were collected in the summer of 2015 from three depths, $1 \pm 0.1$ m ($n = 4$), $2 \pm 0.2$ m ($n = 2$) and $3 \pm 0.2$ m ($n = 3$). Samples were placed on ice in a cooler and subsequently stored frozen and in the dark. The samples were then ground with a mortar and pestle and kept frozen until pigment analysis was performed.
2.3.2 Pigment Extraction and Analysis

Microbialite samples (4 - 6 g) were extracted 3x in acetone under sonication. Hagerthy et al. (2006) reported that certain cyanobacterial pigments can be resistant to extraction under acetone, and that a combination of lyophilization (freeze-drying) and extraction with methanol/acetone/N,N-dimethylformamide (DMF)/water enhanced recovery of cyanobacterial pigments. Acetone is the most common extraction solvent used in pigment studies in part due to the toxicity of DMF, and the potential for chlorin degradation by methanol (Bowles et al., 1985; van Leeuwe et al., 2006). Also, lyophilization is generally avoided unless extraction can commence immediately after due to the rapid degradation of pigments during subsequent storage (Jeffrey et al., 1997). In consideration of the restricted availability of sample material, the more common/safer method of acetone extraction without lyophilization (Roy et al., 2011) was employed in this study. In any case, the very high concentrations of scytonemin extracted indicate that scytonemin is not subject to any inhibitory extraction limitations, potentially due to its existence in the more accessible exopolysaccharide sheath (Garcia-Pichel and Castenholz, 1991).

The samples were sonicated for 15 minutes in a Branson 2510R-MT 100 W sonicator, and then centrifuged for 5 minutes at 515 g in a Sorvall RT 6000B centrifuge. The extracts were then combined and dried under nitrogen gas. All extracts and the final reduced samples were protected from light to avoid photodegradation of pigment extracts.

Pigments were identified using a combination of high performance liquid chromatography (HPLC), photodiode array (PDA) and liquid chromatography-mass spectroscopy (LC-MS). HPLC and PDA analyses were performed on a Thermoscientific Dionex Ultimate 3000 UHPLC with two Waters Spherisorb 3 μM ODS2, 4.6 x 150 mm reverse phase analytical C₁₈ silica columns linked in series. Compound separation was achieved following
method A of Airs et al. (2001), and employed a gradient elution scheme comprising methanol, water, acetonitrile and ethyl acetate (cf. Airs et al., 2001; Method A). Data were processed using Thermo XCalibur software. PDA spectra were gathered over a 300 - 700 nm wavelength range at a 2 nm bunch-width. The LC-MS (Thermo LCQ Fleet, Ion Trap) was used in conjunction with the PDA to compare to known spectra to aid in pigment identification. The LC-MS was operated in positive ion atmospheric pressure chemical ionization (APCI) mode and followed parameters detailed by Airs et al. (2001). Compound quantification was achieved using linear response factors at characteristic wavelengths (chl $a$ 666 nm, scytonemin 388 nm, fucoxanthin 448 nm, $\beta$-carotene 450 nm, chl $b$ 650 nm, and chl $d$ 694 nm) that were either empirically derived from pure standards (Sigma Aldrich and Frontier Scientific), or calculated using molar extinction coefficients for each compound (Jeffrey et al., 1997; Fulton et al., 2018). Integrated peak areas were converted to nanomolar amounts, and then scaled to dry weight of microbialite extracted.

2.3.3 UV and Visible Light Quantification

Photosynthetically active radiation (PAR) measurements were taken using a LI-COR LI-190R Quantum Sensor in conjunction with a LI-250A Light meter and are reported as percent of surface irradiance. UV light attenuation has been shown to be strongly dependent on the concentration of dissolved organic carbon (DOC) in the mixolimnion of freshwater lakes (Morris et al., 1995; Williamson et al., 1996). UV A and UV B profiles were derived based on a model developed using the DOC concentrations for the mixolimnion of 65 glacial lakes in North and South America (Morris et al., 1995; Williamson et al., 1996) (see Eq. 1 – 3) and are also reported as percent of surface irradiation. In equations 1 – 3: $K_d =$ diffuse attenuation coefficient for downwelling radiation (m$^{-1}$); $DOC =$ concentration of dissolved organic carbon (mg/l); $E_{dz} =$
downwelling irradiance (%) at depth \( z \) (m). The average DOC concentration for the mixolimnion of FGL used was determined by Havig et al. (2015, 2017).

1. \( K_{d \text{ UVB}} = 2.09[\text{DOC}]^{1.12} \)

2. \( K_{d \text{ UVA}} = 0.83[\text{DOC}]^{1.16} \)

3. \( E_{dz} = E_{d0} \cdot e^{-K_d \cdot z} \)

2.4 Results

2.4.1 Pigment Identification

In total, 31 pigment compounds from the FGL microbialites were characterized using UV/Vis absorbance and mass spectra (Table 1, Fig. 2), with 27 providing sufficient information to be assigned a structure, and 14 being distinct and not epimers/isomers/allomers of more abundant compounds (Haugan and Liaaen-Jensen, 1994; Airs et al., 2001; Airs and Keely, 2003; Walker et al., 2003; Mawson et al., 2004; Squier et al., 2004a; Roy et al., 2011; Rivera et al., 2014). Scytonemin specifically was identified utilizing its characteristic strong absorption maximum at 388 nm, as well as the diagnostic mass at m/z 545 (M+H)\(^+\) and molecular fragments at m/z 528, 517 and 489 (Fig. 3) (Garcia-Pichel and Castenholz, 1991; Proteau et al., 1993; Squier et al., 2004a). Chlorophyll \( d \) was identified on the basis of the parent ion at m/z 895 (M+H), and absorbance peaks at 454 and 694 nm, with diagnostic red shifted Qy band (Airs et al., 2014).
<table>
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<th>Peak no.</th>
<th>Retention time (min)</th>
<th>Pigment type$^a$</th>
<th>UV/Vis abs. Maxima (nm)$^b$</th>
<th>MS$^1$ (M+H) ion</th>
<th>Other M/Z in MS$^1$</th>
<th>MS$^2$ Fragments$^c$</th>
<th>Principal mass loss</th>
<th>Fragment lost</th>
<th>Compound name$^d$</th>
<th>Structure$^e$</th>
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<td>74.47</td>
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<td>410, 668</td>
<td>887</td>
<td>869, 632, 535</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>28</td>
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<td>Car</td>
<td>448</td>
<td>537</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>278</td>
<td>phytol</td>
<td>Pyropheophytin a</td>
<td>G</td>
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</tbody>
</table>

$^a$: Car is short for carotenoid, Chl/Phy is short for Chlorin or Phytin.
$^b$: Underlined wavelength indicates peak absorbance.
$^c$: ‘’ indicates loss of H$_2$O (-18).
$^d$: Underlined mass indicates principle mass fragment.
$^e$: ep. is short for epimer, ‘’ indicates tentative assignment.
$^f$: See appendix A1 Fig. 1 for molecular structures.
2.4.2 Pigment Abundances

Pigment abundances in general were strongly correlated with microbialite depth. The light shielding pigment scytonemin was more abundant in shallow samples averaging 0.49, 0.15, and 0.02 nmol/g in 1, 2, and 3 m depth samples respectively, following a logarithmic distribution ($r^2 = 0.94; p < 0.01$) (Fig. 4). Light harvesting pigments (chlorophyll a/b and fucoxanthin) showed the opposite relationship and were more abundant in deeper samples (Fig. 4). Chlorophyll a averaged 0.51, 1.14, and 3.29 nmol/g, chlorophyll b averaged 0.01, 0.02, and 0.20 nmol/g, and fucoxanthin averaged 0.14, 0.22, and 0.20 nmol/g at 1, 2, and 3 m depth respectively. β-carotene averaged 0.01, 0.02, and 0.06 at 1, 2, and 3 m depth respectively. Chlorophyll a and b both displayed logarithmic distributions with depth ($r^2 = 0.52$ and 0.69 respectively; $p < 0.1$, and $p < 0.05$ respectively), while fucoxanthin and β-carotene did not exhibit statistically significant trends. Three samples, represented by blue diamonds in Fig. 4(A-E), were initially covered with a thin layer of charophyte algae (Fig. 4F) and were considered separately from non-charophyte covered samples. The charophyte covered samples (S2-1, S3-1md and S2-3ma) contained very little or undetectable amounts of scytonemin when compared to non-charophyte samples, ranging from undetectable to 0.02 nmol/g, with the highest value occurring in the 1 m depth sample S3-1md. Conversely, the 3 m depth charophyte covered sample (S2-3ma), contained the highest concentrations of chlorophyll a (12.62 nmol/g), fucoxanthin (1.67 nmol/g), and β-carotene (0.89 nmol/g) of any sample analyzed.
Figure 2-2. UV/Vis maximum absorbance chromatograms of microbialite acetone extracts. Chromatogram (A) is from 1 m depth and chromatogram (B) is from 3 m depth. Note dramatic difference in scytonemin (K) peak heights between samples. See appendix A1 Fig. 1 for structures and Table 1 for complete compound list.
Figure 2-3. UV-visible light absorption spectrum for scytonemin from Site 3 at 1 m depth showing characteristic maximum absorbance at 388 nm. Inset shows the scytonemin mass spectrum of MS$^1$, with diagnostic base peak at m/z 545 (M+H$^+$) and mass losses for the MS$^2$ ions with m/z 528, 517 and 489.

2.5 Discussion

2.5.1 Pigment Signatures/Broad Chemotaxonomy

The breadth of pigments identified in FGL microbialites are a consequence of a diverse assemblage of organisms living within the mat community. Previous studies have identified cyanobacteria as the dominant class of organism within the microbialites (Thompson et al., 1990; Wilhelm and Hewson, 2012; Shields, 2017). The high $\delta^{13}$C values (-8.5 to -9.5‰) for microbialite biomass reported by Shields (2017), compared with much lower values (-26.0 to -33.2‰) for plankton biomass in the upper 5 m using the same DIC source (Fulton et al., 2018), points toward the dominance of a distinct population of benthic cyanobacteria in the microbialites (Des Marais et al., 1992) different from that in the mixolimnion. High abundance of cyanobacteria conforms with the high concentrations of scytonemin (Fig. 4) (a cyanobacterial
sheath pigment) and low levels of canthaxanthin found in our samples (Table 1, Fig. 2B).

Chlorophyll $a$ and $d$ are also produced in varying abundances by cyanobacteria, but only chlorophyll $d$ is diagnostic, as chlorophyll $a$ is produced by diverse taxonomic classes (Castañeda and Schouten, 2011; Roy et al., 2011; Takaichi, 2011; Loughlin et al., 2013).

Given the abundance of cyanobacteria, the lack of detectable concentrations of myxoxanthrophyll, echinenone and particularly zeaxanthin is anomalous (though we did see low levels of canthaxanthin (Table 1, Fig. 2B)), especially given the presence of *Synechococcus* (Thompson et al., 1990) and *Acaryochloris* (this study), as both are typically associated with high levels of zeaxanthin production (Millie et al., 1993; Duxbury et al., 2009; Roy et al., 2011; Takaichi, 2011; Zhang et al., 2018). The lack of zeaxanthin in the presence of *Acaryochloris* can be explained simply by the low abundance of chlorophyll $d$ observed. Berhendt et al. (2013) reported zeaxanthin/chlorophyll $d$ ratios ranging from ~0.29 - 0.45 in three strains of *Acaryochloris*, and the concentrations of chlorophyll $d$ found in this study were only just above detection limits, meaning that any zeaxanthin derived from *Acaryochloris* is likely below detection limits. Additionally, recent studies of cyanobacterial diversity in FGL microbialites has called in to question the dominance of *Synechococcus* as calcifiers in the mat communities. Wilhelm and Hewson (2012) reported evidence for a dominant suite of endolithic cyanobacterial OTUs distributed throughout the bioherms, with high variability amongst the less prevalent cyanobacteria. Of the amplified DNA across most samples, 5-8% was made up of three OTUs that do not correspond to *Synechococcus* and were the only OTUs to be present in nearly all samples. While this does not preclude the presence of *Synechococcus* in FGL microbialites, it highlights the degree of spatial diversity within the cyanobacterial communities and may provide a partial explanation for the lack of detectable zeaxanthin in our samples.
**Figure 2-4**: Concentration (nmol/g) with microbialite depth profiles: (A) Scytonemin; (B) Chlorophyll a; (C) Fucoxanthin; (D) Chlorophyll b; (E) \( \beta \)-carotene; (F) Examples of charophyte free and charophyte covered samples.
We also have identified pigments which demonstrate the importance of diatoms as constituents of the microbialite organic matter which have previously been shown to be incorporated within the microbialite biofilms in FGL (Thompson et al., 1990; Shields, 2017). The xanthophylls fucoxanthin and other low-abundance accessory pigments such as diadinoxanthin/diadinochrome are produced by diverse algae primarily within the divisions of Heterokontophyta and Haptophyta, including diatoms and bolidophytes (Millie et al., 1993; Castañeda and Schouten, 2011; Roy et al., 2011; Takaichi, 2011) and are likely predominantly sourced from diatoms known to be incorporated into the biofilms studied here (Thompson et al., 1990; Shields, 2017). Fucoxanthin is also used within the light harvesting antennae of diatoms and brown algae in association with chlorophyll $a$ (Alberte et al., 1981; Guglielmi et al., 2005; Veith et al., 2009). β-carotene is produced by an even larger range of photosynthetic algae, and is present in the reactive centers and light harvesting complexes of photosystem I and II, and is thought to serve in both light harvesting and photoprotective capacities in certain organisms (Ben-Amotz and Avron, 1983; Takaichi, 2011). As such, β-carotene is not particularly useful as a specific biomarker, or environmental indicator. Samples that contained chlorophyll $b$, violaxanthin and lutein reflect the contributions of green algal biomass, as these pigments are produced in high quantities by most Chlorophyta including charophytes, which are abundant in FGL (Millie et al., 1993; Castañeda and Schouten, 2011; Roy et al., 2011; Takaichi, 2011). The presence of green algae in FGL microbialites was also observed by Shields (2017) based on lipid biomarker signatures.

Shields (2017) also identified trace amounts of 10-methylhexadecanoic acid, which is a biomarker for sulfate/sulfur reducing bacteria (SRB) (Dowling et al., 1986; Parkes et al., 1993). SRB have been shown to tolerate periodic exposure to oxygen, however they are generally
restricted to anoxic conditions or the oxygen/sulfide interface (Hardy and Hamilton, 1981; Fukui and Takii, 1990; Van Den Ende et al., 1997). The apparent presence of SRB implies some form of vertical community structure/redox gradient within these thrombolitic microbialites. SRB are also commonly associated with purple (PSB) and green sulfur bacteria (GSB) in photosynthetic microbial mats (Overmann and van Gemerden, 2000). However, unlike in the microbial communities within the chemocline and in deeper non-calcifying microbial mats in FGL, there is a distinct lack of detectable carotenoids and bacteriochlorophylls indicative of the presence of PSB and GSB (Meyer et al., 2011; Fulton et al., 2018). We did not detect the PSB specific carotenoid okenone (Caumette et al., 1985; Meyer et al., 2011; Fulton et al., 2018), the GSB specific carotenoid isorenieratene (Liaaen-Jensen, 1978, 2012; Koopmans et al., 1996) or bacteriochlorophylls a, c, d or e (Gloe et al., 1975; Scheer, 1991; Keely, 2006), indicating that the microbial communities within the microbialites did not include anoxygenic photosynthetic sulfur bacteria and are distinct from those deeper in the lake (Fulton et al., 2018).

Lastly, the occurrence of the chlorophyll a derivatives pheophytin a, pyropheophytin a, and hydroxyopheophytin a and their epimers provide evidence for the presence of dead/senescent algal/cyanobacterial tissue within the microbialites extracted (Keely, 2006; Roy et al., 2011). The preservation of relatively high concentration of these pigment derivatives (Fig. 2) suggests that type-I chlorophyll decomposition (the cleavage of macrocycle rings) might have been inhibited by limited oxygen exposure (Keely, 2006). If this dead/senescent tissue is indeed embedded in the most recently calcified mat layers, the calcification process may also account in part for the high levels of scytonemin these samples as scytonemin has been shown to preserve well in overgrown/buried mat surfaces (Garcia-Pichel and Castenholz, 1991).
2.5.2 Scytonemin

The identification of scytonemin in the FGL carbonate microbialites had not been, to our knowledge, previously documented, though its occurrence is not a surprise. Scytonemin has been reported in other microbialites and stromatolites which like those in FGL include dominant populations of colonial and filamentous cyanobacteria (Golubic and Hofmann, 1976; Garcia-Pichel and Castenholz, 1991; Wynn-Williams et al., 1999; Dupraz and Visscher, 2005; Verleyen et al., 2005; Fleming and Castenholz, 2007; Abed et al., 2008; Castenholz and Garcia-Pichel, 2012). Scytonemin is a compound that shields against the effects of UV radiation (Garcia-Pichel et al., 1992), and is produced exclusively by cyanobacteria that exude exopolysaccharide sheaths (Castenholz and Garcia-Pichel, 2012; Soule et al., 2016), which is where scytonemin is concentrated (Garcia-Pichel and Castenholz, 1991; Proteau et al., 1993). Given this function, scytonemin is an unambiguous biomarker for the presence of photosynthesis occurring in an oxidized environment by cyanobacteria. Furthermore, scytonemin is an environmentally stable pigment that is resistant to degradation, and has been shown to be well preserved in Holocene sedimentary samples (Verleyen et al., 2005; Fulton et al., 2012), and potentially in mid-Precambrian stromatolites (Golubic and Hofmann, 1976). Given its role and preservation potential, scytonemin can potentially serve as an indicator of past UV conditions (Fulton et al., 2012; Garcia-Pichel and Castenholz, 1991; Hodgson et al., 2004; Verleyen et al., 2005), as well as an indicator for cyanobacterial communities on other planets such as Mars (Varnali, 2009).

There are factors in addition to UV exposure that influence scytonemin production by cyanobacteria that should be considered when discussing differences in scytonemin concentration with depth. Temperature, oxidative, and osmotic stress have been shown to enhance scytonemin production in cyanobacteria, though only act in magnifying production in
the presence of UV A radiation (Dillon et al., 2002). Osmotic stress, particularly periodic desiccation (Fleming and Castenholz, 2007), has been shown to independently trigger scytonemin production, potentially capitalizing on its high preservation potential in order to facilitate UV A protection and radical scavenging during anhydrobiosis (Matsui et al., 2012). However, because the samples studied here were all at minimum 1 m below the surface of the lake during the height of summer, desiccation is unlikely to be a concern. Furthermore, Havig et al. (2015, 2017) have shown that temperature during July (historically the area’s hottest month on average (NOAA, 2018)) in the upper 3 m of the lake only varies by ~2.5 °C from 0 - 3 m, with a surface max of ~25 - 26 °C, and salinity varies by <0.1 ppt with a max of ~1.0 ppt.

Neither of these factors fall within the range or variability of values thought to induce a strong scytonemin production response in cyanobacteria (Dillon et al., 2002; Abed et al., 2008). Lastly, nitrogen fixation has been shown to induce a 3 - 7x increase in scytonemin in Nostoc punctiforme (Fleming and Castenholz, 2008). Nitrogen fixation does not appear to be significant in FGL microbialites, as δ¹⁵N values for all three locations were 5.3 – 7.7‰ (Shields, 2017), outside the range expected for significant cyanobacterial nitrogen fixation (Bauersachs et al., 2009). It remains that variation in UV A radiation is likely the strongest control on scytonemin production in FGL microbialites.

2.5.3 Chlorophyll d

Chlorophyll d is the principle light harvesting chlorin of Acaryochloris spp., replacing chlorophyll a as the primary electron donor of photosystem I (Tomo et al., 2008; Loughlin et al., 2013) and can comprise up to 99% of its chlorophyll (Miyashita et al., 1997). Acaryochloris spp. are found to be associated with other organisms, such as coral-reef invertebrates (didemnid
ascidians) (Kühl et al., 2005), red algae (Larkum and Kühl, 2005), endolithic crustose coralline algae (Behrendt et al., 2011), and other cyanobacteria such as in stromatolites from shark bay Australia (Goh et al., 2009; Chen et al., 2010) and are widespread throughout marine and terrestrial ecological systems (Kashiyama et al., 2008; Loughlin et al., 2013). In all cases, Acaryochloris spp. are exploiting light environments depleted of visible radiation and enhanced in near infrared light, utilizing the red shifted $Q_y$ band of Chlorophyll $d$ to capitalize on unabsorbed light passing through other organisms (Kühl et al., 2005; Larkum and Kühl, 2005; Loughlin et al., 2013). The presence of chlorophyll $d$ in FGL microbialites indicates the presence of Acaryochloris, and further implies an internal vertical community structuring within these thrombolitic microbialites, with Acaryochloris likely living beneath other cyanobacteria and diatoms/green algae.

2.5.4 Pigment abundances

Pigment abundances in FGL microbialites show a strong dependence on water depth or degree of charophyte cover (Fig. 4). Metagenomic studies using community fingerprinting techniques to characterize the cyanobacterial diversity within the FGL microbialites reveal a similar depth dependency at a site equivalent with this studies site 1; deeper water depths have a higher diversity indicated by more operational taxonomic units within the cyanobacterial communities (see Fig. 5A) (Wilhelm and Hewson, 2012). This raises the question as to how light intensity, or other environmental parameters that vary over a narrow depth range, may affect shallow phototrophic communities and their pigment distributions. Within the upper water column of FGL, primary nutrient and trace metal concentrations and dissolved oxygen are largely invariant from 1 - 3 m over the course of a year (Havig et al., 2015, 2017), and on the
basis of the stability and the we speculate that the most likely control on this diversity is the
effect of UV light on shallow water microbialites.

To combat the harmful effects of UV radiation, photosynthetic organisms increase the
production of photoprotective pigments such as scytonemin and other carotenoids, or
mycosporine like amino acids (MAAs) (Vincent and Roy, 1993; Ehling-Schulz et al., 1997;
Norris et al., 2002; Castenholz and Garcia-Pichel, 2012; Oliveira, 2014). The impact of light
intensity on cyanobacterial pigment distributions is highlighted by the presence and distribution
of scytonemin in mat extracts. Scytonemin is a pigment produced solely by cyanobacteria in
response to exposure to destructive UV radiation (Garcia-Pichel et al., 1992). The concentration
of light-shielding scytonemin shows a non-linear decrease with depth, (Fig. 4) reflecting the
attenuation pattern of UV light in water (Fig. 5A and B) (Fleischmann, 1989; Morris et al., 1995;
Williamson et al., 1996). Derived UV A irradiance levels correlate strongly with scytonemin
Figure 2-5: (A) Measured PAR (400 – 700 nm) intensity (solid line) and UV A (black circles) and B (grey circles) intensities derived from DOC concentration of the mixolimnion (Morris et al., 1995; Williamson et al., 1996; Havig et al., 2015, 2017). All light profiles are plotted as percent of surface irradiance (%). Diversity profiles are based on the Shannon-Wiener Index (SWI) reported by Wilhelm and Hewson (2012) for a site equivalent to site 1 studied here. Graded rectangles show the full range of values reported for each depth, with the darkest region corresponding to the average SWI value; (B) Expanded 1-3 m profile of UV A and B intensities (%), and scytonemin concentration in nmol/g (red circles/dashed line) from charophyte free samples.
concentration ($r^2 = 0.916; p < 0.01; $Fig. A.2$), and demonstrates the community response to UV light, which relaxes with increasing depth. Similar scytonemin distributions in benthic microbial mat communities have been observed in other settings as well (Hodgson et al., 2004; Verleyen et al., 2005; Abed et al., 2008). Cyanobacteria, in general, tend to dominate under more extreme conditions such as high UV irradiance (Golubic, 1991; Abed et al., 2008). The ability to produce scytonemin puts capable strains of cyanobacteria at a distinct advantage over their less UV-tolerant phototroph counterparts, allowing them to utilize otherwise inhospitable habitats. As UV A radiation relaxes at depth, other cyanobacteria that do not produce scytonemin, as well as other phototrophs, become more able to compete with those that do. High light levels also force phototrophic communities to put considerable resources toward the production of light shielding pigments, which may further impact diversity, whilst the overall stability of the environment at depth, coupled with lower UV and visible light flux allows for a greater diversity of phototrophs. Further detailed studies of diversity at sites 2 and 3 and elsewhere in the lake are needed to confirm this relationship, however the strong diversity gradient at site 1 coupled with this studies’ evidence for a UV A control on pigment abundances provides a compelling argument.

The variability seen in scytonemin concentrations from samples collected at the same depth is likely due to several factors. Because the attenuation pattern of UV A light over this depth interval is extremely steep, the uncertainty in depth measurements for our samples ($\pm 0.1$ at $1$ m and $\pm 0.2$ at $2$ and $3$ m) could have a large impact on the amount of UV A light received by a given sample, which in turn would impact the amount of scytonemin produced. For example, calculated UV A intensity varies by $\sim 5.0\%$ over the depth range of $0.9$ to $1.1$ m, and by $\sim 1.2\%$ over the range of $1.8$ – $2.2$ m ($Fig. 5$). Additionally, because scytonemin is produced exclusively by cyanobacteria, lateral variations in the proportion of cyanobacteria to other
organisms within the microbial communities at a given depth could lead to some of the observed variability. Furthermore, a difference in overall irradiance and sunlight experienced over the growing season at each site could impose a control on scytonemin production. Shields et al., (2017) reported that the West bank of the lake (sites 2 and 3) received ~5.0% more sunlight hours than the East bank (site 1) which may have contributed to the variability observed here. Lastly, in several of our samples, scytonemin was in anomalously low concentration or was below the detection limit entirely (blue diamonds in Fig. 4). In these particular samples, low concentrations are attributed to the shading effect of charophyte algae that covered the microbialite surfaces, which acted as “umbrellas” that reduced the need for scytonemin. In spite of the potential for spatial variability, scytonemin concentrations tightly tracked derived UV A irradiance levels with depth (Fig. 5B; Fig. A.2) indicating the potential for scytonemin to be used as a relative water depth/UV A irradiance proxy in ancient microbialites.

The effects of light on pigment distributions in FGL microbialites extends beyond the deleterious effects of UV light on cellular components and processes (Abeliovich and Shilo, 1972; Jagger, 1985). At high light levels, photo-oxidative stress can be induced by the overproduction of triplet oxygen, singlet oxygen radicals and electrons (Krieger-Liszkay, 2005; Vincent and Neale, 2009). A decrease in the concentration of photoprotective pigments with increasing depth is matched by an increase in the concentrations of chlorophyll a. Increased production of chlorophyll a and other light harvesting pigments at lower light intensities is a well described adaptation, expressed in a wide range phototrophic organisms to maximize the utilization of available light (Myers, 1955; Falkowski and Owens, 1980; Raps et al., 1983; Oliveira, 2014). Numerous studies have also shown that cyanobacteria produce less chlorophyll in the presence of high light intensities, even without a concomitant increase in UV radiation
(Leisner et al., 1994; Schagerl and Müller, 2006; Fleming and Castenholz, 2008; Castenholz and Garcia-Pichel, 2012). At high irradiance levels, high chlorophyll concentrations can act as a photosensitizer, particularly in sedentary strains that cannot migrate. In order to increase tolerance to photo-oxidative stress at high light levels, sedentary cyanobacteria will downregulate production of chlorophylls in addition to producing more light shielding pigments (Castenholz and Garcia-Pichel, 2012). Variations in pigment concentrations between samples from the same depth, as well as the lack of a strong trend with depth in fucoxanthin and β-carotene both likely relate to differences in the overall abundance of cyanobacteria relative to diatoms, algae, and other trace phototrophs, or the overall ratio of phototrophic to chemo/heterotrophic organisms within the microbialites.

2.6 Conclusions

Analysis of the pigment distributions within FGL microbialites has revealed a diverse assemblage of cyanobacteria, diatoms, and green algae communities. The concentrations of the photoprotective pigment scytonemin shows a distinct correlation with depth and intensity of UV A light. This coupled with a known increase in cyanobacterial diversity with depth in this system highlights the enormous selective pressure that UV radiation can impose on shallow dwelling microbialite communities. The observed distribution of scytonemin and its correlation with UV A light may also provide a proxy for determining the relative water depth of ancient microbialites in which pigments or their derivatives are preserved.
2.7 Acknowledgements

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CHAPTER 3: CHEMOCLINE COLLAPSE IN LAKE KIVU AS AN ANALOGUE FOR NITROGEN CYCLING DURING OCEANIC ANOXIC EVENTS

Abstract:

One of the outstanding issues in the study of nutrient dynamics during Ocean Anoxic Events (OAEs) is the preservation of strongly $^{15}$N-depleted nitrogen isotope signals, which are not observed in modern environments. The favored mechanism for $\delta^{15}$N values below 0‰ is the influence of sub-chemocline-derived ammonium on primary production, but there is are few modern analogues available for testing this hypothesis, due to the overall well oxygenated state of the modern oceans. Here, we present sedimentary $\delta^{13}$C$_{org}$ and $\delta^{15}$N$_{bulk}$ data, as well as pigment biomarker ratios, from the sediments of Lake Kivu, a meromictic lake in East Africa. We focus on a series of intervals of organic carbon enrichment that are interpreted to have been the result of water column mixing events or limnic eruptions. Sediment $\delta^{13}$C$_{org}$ and $\delta^{15}$N$_{bulk}$ values display sharp negative excursions of up to ~6 and 4‰ at the base of sapropel layers highly enriched in organic matter. These negative excursions provide evidence for the mixing $^{13}$C-depleted dissolved inorganic carbon and high concentrations of ammonium derived from below the chemocline. Additionally, we utilize the ratio of zeaxanthin:chlorophyllone which demonstrates enormous fluctuations in the ecological balance of the water column, consistent with a shallowing of the euphotic zone. Further evidence for the shallowing of the chemocline during sapropel deposition is found in the presence of bacteriochlorophyll derivatives produced by phototrophic sulfide oxidizers. This study provides a potential analogue for the development of strongly depleted $\delta^{15}$N$_{bulk}$ values in black shales of OAEs.
3.1 Introduction:

Instances of widespread ocean anoxia have occurred throughout the Phanerozoic, with the most widespread and prolonged being the Ocean Anoxic Events (OAEs) of the Mesozoic (Jenkyns, 2010; Karakitsios et al., 2010; Schlanger et al., 1987; Schlanger and Jenkyns, 1976; Takashima et al., 2006). These events were the result of global-scale changes in Earth’s baseline geochemical state (Jenkyns, 2010) and are expressed in the geologic record by the extensive deposition of organic-matter enriched rocks (Schlanger et al., 1987; Schlanger and Jenkyns, 1976). Spatially expansive deoxygenation events are also commonly associated with elevated rates of marine extinction throughout the Phanerozoic, such as the Frasnian-Famennian Biotic Crisis (Bambach, 2006; Racki, 2005), the Permian-Triassic mass extinction (Erwin, 1994; Wignall and Hallam, 1992), the Early Toarcian (T-OAE) (Caruthers et al., 2013; Harries and Little, 1999), the Cenomanian-Turonian (OAE 2) (Harries, 1993; Jablonski, 1991) and the Paleocene-Eocene Thermal Maximum (Speijer et al., 2012), among others. Many of these events were associated with increases in the concentrations of greenhouse gases and associated warming (Barclay et al., 2010; Jenkyns, 2010; Leckie et al., 2002; Schlanger and Jenkyns, 1976).

In an analogous fashion, due to anthropogenic greenhouse gas emissions and warming the areal extent of seasonal hypoxic ‘dead zones’ is increasing in the modern ocean (Diaz and Rosenberg, 2008) and the average concentration of dissolved oxygen in the ocean shows a decline over the last 60 years (Breitburg et al., 2018; Schmidtko et al., 2017). The study of ancient periods of widespread deoxygenation can provide perspective on modern warming and the spread of ocean anoxia (Jenkyns, 2010). Such investigations can also help constrain the effects of deoxygenation on marine ecosystems (Payne et al., 2016) and nutrient cycling, as both
nitrogen and phosphorous are processed differently under anoxic conditions (Benitez-Nelson, 2000; Galbraith and Sigman, 2008; Higgins et al., 2012; Van Cappellen and Ingall, 1994). The unique nitrogen geochemistry that characterizes intervals of past anoxia, specifically, the anomalously low $\delta^{15}\text{N}$ values observed in the black shales associated with Mesozoic OAEs, and other events such as the PETM (Dumitrescu and Brassell, 2006; Higgins et al., 2012; Jenkyns et al., 2001; Junium et al., 2018a, 2018b, 2015; Junium and Arthur, 2007; Ruvalcaba Baroni et al., 2015), is generally not observed in the modern ocean or extant lake basins. Because today’s oceans are overall still well-oxygenated, there are few modern analogue systems available for detailed study of the mechanisms that allowed for preservation of $^{15}\text{N}$-depleted signals in ancient sediments.

Here we present sedimentary $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ and pigment biomarker data from Lake Kivu, in the East African Rift Valley, that provide context and a modern analogue for the nitrogen biogeochemical record of Oceanic Anoxic Events. Lake Kivu is permanently stratified with anoxic deep waters, and because of its potential to emit lethal concentrations of CO$_2$ into the densely populated surrounding area, its physical and chemical limnology (Degens et al., 1973; Schmid et al., 2005; Tassi et al., 2009), biogeochemistry (Morana et al., 2015b; Muvundja et al., 2009; Sarmento et al., 2012, 2006), and microbial ecology (Bhattarai et al., 2012; Llirós et al., 2010; Morana et al., 2016, 2015a; Roland et al., 2016; Zigah et al., 2015) have been intensively studied. Lake Kivu has also experienced a number of volcanic and climatic perturbations over the last ~10 kyr which have disturbed its stratification, ecology, and biogeochemistry (Ross et al., 2015; Zhang et al., 2014); accordingly it is an intriguing study area for investigating the effects of chemocline destabilizations on nutrient cycling and the associated stable isotope signals.
On short time scales, the carbon isotope composition of organic matter ($\delta^{13}C_{\text{org}}$) can act as a tracer of local carbon cycling dynamics, while the nitrogen isotope composition of bulk sediments ($\delta^{15}N_{\text{bulk}}$) can serve as a proxy for nutrient dynamics, sources of biologically available nitrogen, and provide information on how redox processes affect the isotopic composition of reactive nitrogen reservoirs. Together, $\delta^{13}C_{\text{org}}$ and $\delta^{15}N_{\text{bulk}}$ studies can help to reconstruct the interplay of regional water-column redox structure and primary producer communities.

Pigments are lipids that interact with and absorb specific wavelengths of light, depending on their structure and electronic properties. In microbial organisms, pigments are directly associated with photosynthesis (e.g. chlorophyll $a$) or serve a variety of roles in support of photosynthesis, including photoprotective roles such as UV screening (Castañeda and Schouten, 2011; Ehling-Schulz et al., 1997; Roy et al., 2011), the dissipation of excess absorbed energy through the xanthophyll cycle (Goss and Jakob, 2010), and can serve as antioxidants (Latifi et al., 2009). In many cases, pigments can be diagnostic of the presence of specific classes of organisms; for example, notable differences in the structures of many bacteriochlorophylls allow for positive identification of anoxygenic photoautotrophs from ancient systems (e.g. Keely, 2006). The appearance of specific biomarker pigments, as well as the relative concentrations of photoprotective and light harnessing pigments (Kana et al., 1988; Leisner et al., 1994) can therefore be used to help characterize the locus of primary production and the depth of the chemocline through time in a given system.

On the basis of the results presented here, we propose that past intervals of chemocline instability and water column mixing led to the delivery of isotopically depleted dissolved inorganic carbon (DIC) and ammonium ($\text{NH}_4^+$) along with phosphate ($\text{PO}_4^{3-}$) to surface waters. The utilization of these newly available reservoirs of nutrients by primary producers at the
surface led to the deposition of strongly $^{13}$C and $^{15}$N-depleted organic matter in sediments immediately after mixing events. This process may provide a mechanistic link for the influence of chemocline-derived nutrients on strongly depleted sedimentary stable isotope signatures in ancient deposits.

![Maps](image)

**Figure 3-1:** Left – Map of Eastern Africa. Dark grey dashed lines show general position of fault lines. Right – Map of Lake Kivu, red dot shows sample site.

### 3.2 Study Area:

Lake Kivu is a 482-meter-deep meromictic lake situated within the Western branch of the East African rift system and straddles the border of the Democratic Republic of Congo and the Republic of Rwanda (Fig. 1). The East African rift system is a seismically active zone of lithospheric extension, and contains a number of rift basins separated by accommodation zones.
Lake Kivu is the highest of these lakes, with a surface elevation ~1463 m above sea level (Degens et al., 1973). The lake has undergone a series of lake level changes throughout the past ~15 kyr, the most extreme of which occurring ~12 kyr ago when lake levels rose upwards of 400 m (Wood and Scholz, 2017). A number of studies attribute the dramatic rise at ~12 kyr and modern lake level to the impoundment of the north end of the lake by a rapid expansion of the Virunga Volcanic Province (Degens et al., 1973; Haberyan and Hecky, 1987; Ross et al., 2014), which dammed the northern outflow and led to the current drainage to the south along a border fault to Lake Tanganyika through the Rusizi River (Fig. 1)(Degens et al., 1973). Wood and Scholz (2017) however argue for transgression related to changing climatic conditions, and a more gradual expansion of the Virunga Volcanic Province to accommodate the lake level rise.

The limnology of Lake Kivu is complex; it is meromictic and has three principle density layers with boundaries situated at ~65 and 255 m. Stratification is maintained by hydrothermally-sourced, saline hot springs located at depth, which support a 4.5 g/l density contrast between surface and deep waters (Bhattarai et al., 2012; Schmid et al., 2005) (Fig. 2). Below the mixed layer (upper ~65 m) resides high concentrations of carbon dioxide (CO₂), up to 0.10 mol/l, and economically viable concentrations of methane (CH₄), up to 0.02mol/l. In total this equates to ~300 km³ and 60 km³ of gas respectively at STP (Schmid et al., 2005). The CH₄ is thought to be derived primarily from microbial methanogenesis within the anoxic layers of the water column and sediments through the utilization of both acetate and geogenic H₂ (Bhattarai et al., 2012; Pasche et al., 2011), and the CO₂ from active volcanism within the basin (Deuser et al., 1973; Tedesco et al., 2010).
The development of meromixsis and the high concentrations of CO$_2$ support an extremely steep pH gradient, with acidic deep waters achieving a pH of 6 but with surface waters reaching a pH of ~9, which currently support active carbonate precipitation (Bhattarai et al., 2012; Schmid et al., 2005; Tassi et al., 2009). Today, the top of the chemocline is situated at ~65 m, and limits convective mixing to above 65 m (Schmid and Wüest, 2012). Here we will operationally define the chemocline as the point below which sulfide (H$_2$S) and NH$_4^+$ concentrations first rise rapidly, which is also coincident with the epilimnion–metalimnion boundary (Fig. 2). The chemocline is characterized by sharp redox and chemical gradients, with the deep layers of the lake hosting H$_2$S, NH$_4^+$, and phosphate (PO$_4^{3-}$) concentrations of up to 350, 4000, and 184 µM respectively (Schmid et al., 2005; Schmid and Wüest, 2012; Tassi et al., 2009). The epilimnion (upper-most, lowest density layer, Fig. 2) of the lake evolves seasonally due to cooling-induced convection and wind forcing, with deeper mixing in the dry season allowing for the delivery of nutrients from in/near the chemocline (Sarmento et al., 2006).
**Figure 3-2:** Water column structure of Lake Kivu. E denotes epilimnion, M – metalimnion, H – hypolimnion. Salinity and temperature are represented by S and T respectively. For oxygen content, solid black line shows rainy season profile (R) and dashed line shows dry season profile (D). Salinity and temperature data are from Schmid et al. (2005), and oxygen concentration data are from Sarmento et al. (2006). Vertical scale is in meters (m) and exaggeration is ~60x.

**3.3 Methods:**

**3.3.1 Core collection:**

Core samples were collected in March of 2013 aboard the R/V Kilindi from the Northern Basin of Lake Kivu. Core 13-13A-1K was collected at 29° 13’ 37.884” E, 1° 51’ 3.384” S, and at 427 m water depth (Fig. 1). Coring was carried out utilizing a modified Kullenberg piston coring system equipped with a 380 kg driving weight, in 7 cm diameter polycarbonate tubes (Wood and Scholz, 2017; Zhang et al., 2014). Cores were subsequently stored at 3°C until split and sampled. Samples for isotopic analyses were taken at 0.5 cm intervals from 17-43 cm (37-63 cmblf) depth interval of KIVU13-13A-1K section 2 (n = 48), and larger samples for pigment analysis were taken at 1.0 cm intervals (n = 12) and subsequently frozen. Samples were then lyophilized and crushed with a mortar and pestle to homogenize the sediments, and sample powders used for isotope analyses were acidified with 3 N hydrochloric acid, rinsed with deionized water and re-lyophilized prior to analysis.

**3.3.2 Isotopes:**

Nitrogen and carbon isotopic analyses of decarbonated sediments were performed in the Syracuse University Geobiology, Astrobiology, Paleooclimate, and Paleoceanography (GAPP) Lab using an Elementar Isotope Cube elemental analyzer (EA) coupled directly to an Isoprime 100 isotope ratio mass spectrometer (IRMS) using conventional techniques for EA-IRMS.
Samples were weighed into tin cups, evacuated, and sparged with Ar to remove interstitial N₂. EA conditions were as follows: helium purge was set for 30 seconds, oxidation and reduction reactor temperatures were 1100 °C and 650 °C, respectively; helium carrier gas flow was 230 ml/min; and the O₂ pulse was set for 60 seconds. International reference materials IAEA N1 ammonium-sulfate [δ¹⁵N = +0.4 ‰]; N2 ammonium-sulfate [δ¹⁵N = +20.3 ‰]; NIST 1547 peach leaves [δ¹⁵N = +2.0 ‰; δ¹³C = −26.0 ‰]; ANU Sucrose [δ¹³C = −10.45 ‰] and Messel Oil Shale (in house standard [δ¹⁵N = +7.0 ‰]) were used in concert with sample material for reference calibrations. The resulting blank corrected sample and standard data were corrected to accepted values for the reference materials using the correction scheme described in Coplen et al., (2006). Reproducibility for samples and standards for δ¹⁵N was better than +/- 0.2 ‰ but is reported as such to reflect the reported nitrogen isotopic composition of the reference materials (+/- 0.2 ‰). Reproducibility for samples and standards for δ¹³C was +/- 0.1 ‰. Carbon isotope values are presented relative to Vienna Pee Dee Belemnite (VPDB), and nitrogen isotope values are relative to atmospheric N₂.

3.3.3 Pigments:

Samples were extracted in acetone by sonication in a chilled water bath until resulting extracts were clear. The extracts were filtered through defatted cotton wool, combined and dried in a TurboVap under nitrogen gas stream (e.g. Junium et al., 2011). All extracts and the final reduced samples were protected from light to avoid photodegradation of pigment extracts. Pigments were identified using a combination of high-performance liquid chromatography (HPLC), photodiode array (PDA) and liquid chromatography-mass spectroscopy (LC-MS). HPLC and PDA analyses were performed on a Thermoscientific Dionex Ultimate 3000 UHPLC
with two Waters Spherisorb 3 µM ODS2, 4.6 x 150 mm reverse phase analytical C\textsubscript{18} silica columns linked in series. Compound separation was achieved following method A of Airs et al. (2001), and employed a gradient elution scheme comprising methanol, water, acetonitrile and ethyl acetate (cf. Airs et al., 2001; Method A). Data were processed using Thermo XCalibur software. PDA spectra were gathered over a 300 - 700 nm wavelength range at a 2 nm bunch-width. The LC-MS (Thermo LCQ Fleet, Ion Trap) was used in conjunction with the PDA to compare to known spectra to aid in pigment identification. The LC-MS was operated in positive ion atmospheric pressure chemical ionization (APCI) mode, and conditions were as follows: capillary temperature 150˚C, APCI vaporizer temperature 450˚C, discharge current 5µA, and sheath gas flow 60 (arbitrary units) as described by Airs et al. (2001).

Pigments were quantified based on specific wavelengths (660 nm for chlorophyllone, 446 nm for lutein, and 450 nm for zeaxanthin), and then scaled to TOC. The zeaxanthin:chlorophyllone ratios were scaled to the relative zeaxanthin:lutein ratio to account for the contribution of green algae to the chlorophyllone pool. The diatom related pigments fucoxanthin and diadinoxanthin/diadinochrome were not observed/were below detection limits in these samples, and so a similar scaling for the contribution of diatom biomass could not be performed. Given the presence of high numbers of diatoms in the modern lake (Sarmiento et al., 2006) and the studied sediments, the detection of the pigments fucoxanthin and diadinoxanthin/diadinochrome would be expected as they are typically present in high abundance across most clades of diatoms (Roy et al., 2011). However, both fucoxanthin and diadinoxanthin typically have low accumulation rates in sediments compared to other pigments (Itoh et al., 2007) potentially due to the presence of the 5’6-epoxide ring (Repeta and Gagosian, 1987; Steenbergen et al., 1994) which can allow for degradation to loliolide relatively quickly
even under anoxic conditions (ca. kyrs) (Damsté and Koopmans, 1997). Fucoxanthin can also degrade to fucoxanthinol through the hydrolysis of the acetate group on the 3,5,5-trimethylcyclohexyl ring (Repeta and Gagosian, 1987). We find some potential evidence for fucoxanthinol (617 [M+H] ion) in select mass spectra but the abundances were too low to make a definitive identification.

3.4 Results:

3.4.1 Sedimentologic description

Sediments within the sampled interval ranged from dark-reddish brown tan, sub-mm-scale laminated, organic matter-rich muds to light tan, massive diatomaceous muds (Fig. 3). Sampling was focused on the first occurrences of the prominent dark-reddish brown, organic matter-rich muds (further referred to as sapropels) that are common constituents of the recent Lake Kivu sedimentary record (Ross et al., 2015; Wood and Scholz, 2017; Zhang et al., 2014). The layers immediately below the sapropels have been characterized as either hyperpycnites (Zhang et al., 2014) or volcanic tephra (Ross et al., 2015) in cores from nearby locations (Fig. 2). The basal-most layer of the sapropels is particularly rich in diatoms, and is characterized as a diatomite (Zhang et al., 2014).

3.4.2 Bulk geochemical parameters:

Total organic carbon wt. % (TOC) was elevated through most of the core, averaging 13.8 % and ranging from 2.5 – 21.8 % with the highest concentrations occurring in the sapropel layers, and the lowest occurring in the hyperpycnite/tephra layers immediately below them. Total nitrogen wt. % (TN) averaged 0.9 % and ranged from 0.2 – 1.6 %, and displayed maxima and minima in
the same samples as TOC (Fig. 3). C/N ratios ranged from 12.7 – 30.6 with the highest ratios occurring in the lowermost sediments prior to sapropel deposition (Fig. 1-E in appendix A2).

3.4.3 Carbon and nitrogen stable isotopic composition

$\delta^{13}C_{\text{org}}$ values ranged from -31.0 – -22.9 ‰ with an average of -25.5 ‰. Sapropel layers display significant offsets of up to ~6 ‰ at their onset, with the oldest sapropel having the largest offset (ME 1 in Fig. 3). Background values outside of the negative excursions lie around -24.5 ‰. $\delta^{15}N_{\text{bulk}}$ values averaged 2.5 ‰ and ranged from -1.7 – 7.8 ‰ and also displayed radical fluctuations during sapropel deposition. At the initiation of sapropel events $\delta^{15}N_{\text{bulk}}$ dropped by up to ~3 ‰, and then increased rapidly toward the top of the sapropels by up to ~9 ‰, again with the oldest sapropel having the largest offset (Fig. 3). The $^{15}$N-enrichment at the top of the sapropels is consistent with other lower resolution studies from elsewhere in the basin (Ross et al., 2015).
Figure 3-3: TOC, isotope, and pigment ratio data from Lake Kivu. Horizontal dashed line denotes the initiation of sapropel deposition, vertical dashed lines denote background isotopic averages. Vertical scale is section depth, overall depth of core depicted is 35-65 cm blf. Horizontal grey bars denote potential mixing events (ME 1-3). Note the logarithmic scale used for the zeaxanthin:chlorophyllone ratio (Zeax:Chl).
3.4.4 Geochemical Relationships

Values of $\delta^{13}C_{\text{org}}$ and TOC showed no significant Pearson’s correlation ($r = 0.03$, $p = 0.83$) indicating that there was no effect of differential preservation or diagenesis of organic matter on the $\delta^{13}C_{\text{org}}$ signal (Fig. 1-A in appendix A2), though the maximum TOC values typically occurred ~coevally or slightly after minimum $\delta^{13}C_{\text{org}}$ values, along with the minimum and maximum $\delta^{15}N_{\text{bulk}}$ values. The $\delta^{13}C_{\text{org}}$ data show a significant correlation with C/N ratios ($r = 0.39$, $p < 0.01$), but this was mostly driven by a few extreme end members, specifically, the strongly $\delta^{13}C_{\text{org}}$ values from within the sapropels (Fig. 1-B in appendix A2). Values of $\delta^{15}N_{\text{bulk}}$ showed no significant correlation with TOC or TN ($r = 0.05$, $p = 0.76$ and $r = 0.22$, $p = 0.14$ respectively) and thus are likely minimally affected by inorganic nitrogen bound to terrestrially-derived clays (Fig. A1-C,D) (Calvert, 2004). There was also a significant correlation between $\delta^{15}N_{\text{bulk}}$ and C/N ratios, but this was again driven primarily by four high C/N ratio samples in the sediments prior to sapropel deposition (Fig 1-E in appendix A2).

3.4.5 Pigments:

HPLC-UV/Vis and MS$^n$ analysis of acetone extracts revealed the preservation of a diverse suite of photosynthetic and accessory pigments in the Lake Kivu sediments, with the majority of them carotenoids and their derivatives (Fig. 4). The carotenoids zeaxanthin and lutein were by far the most abundant pigments in all samples. Zeaxanthin:lutein ratios and zeaxanthin:chlorophyllone ratios were highest in and around the lower-most sapropel layer, up to 6.3 and 54,000 respectively (Fig. 3). The organic rich layers also have bacteriochlorophyll derivatives, indicative of the presence of green and purple sulfur or non-sulfur bacteria.
3.5 Discussion:

3.5.1 Carbon:

The $\delta^{13}C$ of sedimentary organic matter in aquatic systems is dependent on a number of interrelated parameters: $pCO_2$ (Freeman and Hayes, 1992; Hollander and McKenzie, 1991), organic matter source (Meyers, 1994), microbial ecology/the biosynthetic pathway of carbon fixation (House et al., 2003; Hügler and Sievert, 2011), the $\delta^{13}C$ of DIC being utilized for organic carbon production (Fogel and Cifuentes, 1993), and the extent of heterotrophy which can lead to an isotopic enrichment in preserved sedimentary organic matter. The relative influence of terrestrial organic carbon on the isotopic composition of sediments in an enclosed landlocked basin is a reasonable concern, as a large contribution of allochthonous organic matter could overprint or shift the isotopic signal associated with autochthonous water column production.
However, the lake is currently net-autotrophic (gross primary productivity > ecosystem respiration) and the organic carbon in the sediment has been determined to be predominantly autochthonous, with very little organic matter entering the lake through riverine influx (Borges et al., 2014; Morana et al., 2015b), likely due in part to the small catchment size of the basin (~4940 km²) relative to its surface area (~2370 km²) (Muvundja et al., 2014).

In the modern lake ~65 – 75% of the DIC used for primary production is derived from geogenic CO₂ and methanogen-derived CH₄ diffusing upwards from deeper in the lake, where there is a net loss of CO₂ to the atmosphere (Pasche et al., 2011). The isotopic composition of the DIC follows step wise depletions from a δ¹³C of ~4‰ in the epilimnion to ~2‰ in the metalimnion (intermediate density layer) and ~2‰ in the hypolimnion (basal-most, highest density layer) (Fig. 5A) (Tassi et al., 2009). Particulate organic carbon in the epilimnion of the lake has an average isotopic composition of ~23‰, but displays a strong depletion starting just above the chemocline to as low as ~42‰, and returns to ~27‰ below the chemocline (Borges et al., 2014). The strong depletion observed near the chemocline is the result of the utilization of ¹³C-depleted DIC and specifically methane, with an isotopic composition as low as ~60‰ (Borges et al., 2014). The dissolved methane constitutes an important carbon and energy source (up to 38%) for the heterotrophic and autotrophic communities in the lake (Zigah et al., 2015). The average isotopic composition of ~27‰ of sinking particulate organic carbon below the region of autotrophic production aligns well with the background δ¹³C values observed in the studied core (Fig. 3). Accordingly, the negative excursions observed in the sapropel intervals may reflect a change in the relative balance of the source of DIC used for the bulk of primary production, to being more influenced by the ¹³C-depleted DIC and methane reservoirs below the chemocline in the hypolimnion of the lake. A distinct change in the isotopic composition of the
DIC source is a particularly viable explanation, given the cooccurrence of negative $\delta^{13}\text{C}_{\text{org}}$ excursions with the diatomite layers at the base of the sapropels (Fig. 3-3). Protracted diatoms blooms have traditionally been associated with a positive shift in the $\delta^{13}\text{C}$ composition of bulk organic matter as the extensive rapid productivity draws down and progressively enriches the DIC reservoir in $^{13}\text{C}$ (Deuser, 1970). The lack of enrichment within the diatomite layers relative to background implies that the isotopic composition of the DIC source likely shifted enough to offset any enrichment from diatom blooms.

Previous studies have described past water column mixing events in Lake Kivu driven either by hyperpycnal flows (Zhang et al., 2014) or subaquatic volcanism (Ross et al., 2015) that destabilized the stratification and chemocline in the lake, and allowed upward mixing of nutrient-replete deep-waters to the surface, similar to the more recent limnic eruptions experienced by Lakes Nyos (Kling et al., 1987) and Monoun (Sigurdsson et al., 1987). These mixing events are demarcated by layers interpreted to be hyperpycnites (Zhang et al., 2014) or volcanic tephra (Ross et al., 2015) where the normally elevated background TOC content decreases, generally followed by a diatomite layer, and a sapropel layer of elevated TOC concentrations of up to ~20% in this study, and up to ~35% in nearby cores (Ross et al., 2015). This pattern is observed at multiple stages within the studied core interval (Fig. 3). The oscillations in TOC co-occur with the negative excursions in $\delta^{13}\text{C}_{\text{org}}$, implying that the excursions are related to the mixing events (Fig. 3). On the basis of the $\delta^{13}\text{C}_{\text{org}}$ and TOC patterns observed here, we identify three to four potential mixing events in the studied section, with the two most obvious showing the greatest $^{13}\text{C}$-depletions and TOC fluctuations (ME 1 and 2 in Fig. 3). The mechanism for this connection is relatively straightforward: breakdown in stratification leads to upwelling of the isotopically depleted DIC, the oxidation of dissolved organic matter and methane, as well as the delivery of
NH₄⁺ and phosphate present in the meta/hypolimnion of the lake to the epilimnion where it is used by surface phytoplankton, leading to the production of strongly ¹³C-depleted biomass (Fig. 5B). As stratification is re-established (Fig. 5C) the δ¹³Corg of the sediments returns to background averages. This provides a direct, albeit short-lived, analogue for the model of ¹³C-depleted DIC utilization during localized black shale deposition in the Toarcian (Küspert, 1982), the Late Permian Mass Extinction (Mettam et al., 2017), and the Frasnian-Famennian Biotic Crisis of the Late Devonian (Uveges et al., 2018), where similar negative δ¹³Corg excursions are observed associated with organic rich shales.

Assuming that the paired light-dark sediment layers in the core can be interpreted as annual varves, a visual varve count indicates that the lower-most (and largest) sapropel is ~65 years in duration. Average sedimentation rate from core 12-19A of Zhang et al. (2014) which was approximately 25 km to the South, and at 352 m water depth is ~0.55 ± 0.36 mm/yr, however this is including a number of turbidites that were likely deposited in discrete events and utilizing roughly correlative ¹⁴C dates from other cores. Using two dates from the same core in a section with minimal influence from turbidites and mostly comprised of laminated dark-greenish grey mud yields a sedimentation rate of ~0.29 mm/yr or ~3.5 yr/mm, which is likely closer to what was experienced post mixing events in the studied section. The lower-most sapropel is ~2.5 cm thick and using the sedimentation rate above, an estimated duration of about 90 years can be derived. This agrees moderately well with the varve count, and tends to indicate that the longest sapropelic deposition associated with the inferred mixing events was less than 100 yrs.
Figure 3-5: Mixing scheme - (A) Strong stratification similar to modern state. Chemical data from Tassi et al. (2009) and Schmid et al. (2005); (B) During lake mixing event and chemocline collapse; (C) Start of stratification recovery period. Vertical exaggeration ~70x. Graded region indicates oxygen-sulfide transition zone. Isotope values in white boxes indicate surface sediment $\delta^{13}$C$_{org}$ and $\delta^{15}$N$_{bulk}$ compositions during each phase.

3.5.2 Nitrogen:

The $\delta^{15}$N$_{bulk}$ of sediments reflects the relative balance of a suite of microbially mediated processes that act upon nutrient nitrogen species (Canfield et al., 2010; Galbraith and Sigman, 2008). Nitrogen is added to most aquatic systems either through riverine delivery of nitrate ($\text{NO}_3^-$) or directly from fixation of $\text{N}_2$ by diazotrophic ($\text{N}_2$ – fixing) cyanobacteria. The rate of diazotrophy is generally controlled by $\text{PO}_4^{3-}$ availability (Moutin et al., 2008, 2005; Polyviou et al., 2015), which is largely derived from weathering (Benitez-Nelson, 2000), but can be efficiently recycled in anoxic systems (Adams et al., 2010; Ingall and Jahnke, 1997; Ingall et al., 1993; Van Cappellen and Ingall, 1994). Anoxic/dysoxic regions of the water column host a series of oxygen sensitive processes which typically act to remove nitrogen from the DIN pool.
Both nitrate reduction (denitrification), and anaerobic \( \text{NH}_4^+ \) oxidation (anammox) serve as a terminal sink for nutrient nitrogen by converting it to either \( \text{N}_2 \) or \( \text{N}_2\text{O} \) (Brandes et al., 2007; Canfield et al., 2010). Each of these processes is associated with an intrinsic normal, kinetic isotope effect in which the lighter isotope \(^{14}\text{N}\) reacts faster and is thus preferentially utilized in a given reaction. Therefore, the processes that act to add nitrogen to a system (N-fixation) will lower the \( \delta^{15}\text{N} \) composition of the nitrate/DIN pool, while processes that act to remove nitrogen (nutrient uptake, denitrification, anammox, etc.) will raise the \( \delta^{15}\text{N} \) composition of the residual nitrate/DIN pool. Sedimentary organic matter \( \delta^{15}\text{N}_{\text{bulk}} \) values can be reliable tracers of sub-euphotic zone nitrate, and are therefore used to track the evolution of nitrogen cycling processes at a particular site through time (Robinson et al., 2012; Tesdal et al., 2013; Thunell et al., 2004). In modern or recent systems like Lake Kivu, the conditions that alter primary \( \delta^{15}\text{N}_{\text{bulk}} \) values (time, high temperatures, abundant oxygen over > 1000 m depth) are generally absent.

In modern Lake Kivu, the nitrogen isotopic composition of particulate OM varies seasonally, with relatively enriched values (~4‰) occurring in the dry season during shallow mixing events producing a diatom dominated ecosystem, and relatively depleted values (~0-2‰) occurring during the rainy season in a cyanobacteria dominated ecosystem that likely includes diazotrophs (Morana et al., 2015b). This range in \( \delta^{15}\text{N} \) values is in agreement with the average background \( \delta^{15}\text{N}_{\text{bulk}} \) of +2‰ observed in the studied core (Fig. 3). \( \text{NH}_4^+ \) is present in high concentrations in the deep waters of Lake Kivu (Fig. 5) (Schmid et al., 2005; Tassi et al., 2009), and this is a common feature in other strongly stratified water columns (Fulton et al., 2012; Havig et al., 2017; Velinsky and Fogel, 1999; Voß et al., 1997). This \( \text{NH}_4^+ \), as well as the high concentrations of phosphate in the deeper layers of the lake supply ~75% of the DIN and ~90% soluble reactive phosphorous load to the surface waters through upward advection and weak
mixing during the dry season, with the balance being provided by riverine input and diazotrophy (Muvundja et al., 2009; Pasche et al., 2012; Sarmento et al., 2006). DIN in the epilimnion is predominantly nitrate due to the stepwise oxidation of NH$_4^+$ diffusing through the chemocline (Llirós et al., 2010; Morana et al., 2016; Roland et al., 2017), with discrete intervals of elevated nitrite concentrations associated with the locus of ammonia oxidation (Llirós et al., 2010).

During the mixing events described above, upwelling deep waters would deliver abundant NH$_4^+$ and phosphate which are trapped in the hypolimnion of the lake, to the epilimnion (Fig. 5B), driving enhanced productivity near the surface. In the presence of both NH$_4^+$ and nitrate, phototrophic organisms will utilize NH$_4^+$, as it is already in the most biologically useful oxidation state (Eppley et al., 1969), and ambient NH$_4^+$ inhibits the expression of the genes that regulate the proteins used in the nitrate assimilation process (Flores and Herrero, 2005). Therefore, NH$_4^+$ can have a significant contribution to the particulate nitrogen pool even at lower relative concentrations (Harrison et al., 1996; Pennock et al., 1996; Probyn et al., 1996). NH$_4^+$ uptake also has a much larger assimilatory fractionation than does nitrate, and incomplete utilization can result in the production of much more $^{15}$N-depleted biomass given equal starting compositions (Hoch et al., 1994, 1992; Pennock et al., 1996; Waser et al., 1998). The greater prevalence and utilization of NH$_4^+$ during mixing events likely drove the $^{15}$N-depletions observed in coeval sediments (Fig. 3, 5B), and is directly analogous to the mechanism proposed for past instances of organic-rich shale deposition with depleted $\delta^{15}$N$_{bulk}$ values during the Late Devonian (Uveges et al., 2018), the T-OAE (Jenkyns et al., 2001), OAE 2 (Higgins et al., 2012; Junium and Arthur, 2007; Ruvalcaba Baroni et al., 2015; Junium et al., 2018), and the PETM (Junium et al., 2018a).
Directly above the negative shifts in $\delta^{15}N_{\text{bulk}}$, the isotopic composition of the sapropels undergoes strong shifts of up to $\sim$9‰, to values as high as +7.8 ‰ (Fig. 3). This rapid shift to strong $^{15}$N-enrichment likely reflects the re-establishment of stratification (Fig. 5C), and the quantitative utilization of the enriched residual pool of NH$_4^+$ above the chemocline, and then a return to background concentrations and a nitrate dominated epilimnion (Fig. 5A). Alternatively, the partial down-mixing of diluted oxygen to deeper waters, and presence of high nutrient levels could lead to an increase in the highly fractionating processes of water column denitrification and annamox, both of which require low levels of oxygen to proceed (Brandes et al., 2007; Brunner et al., 2013; Thamdrup and Dalsgaard, 2009; Ward et al., 2009), similar to what is proposed for large positive shifts associated with the T-OAE (Jenkyns et al., 2001), and during glacial-interglacial transitions in the Black Sea (Quan et al., 2013).

An additional control on the isotopic composition of dissolved NH$_4^+$ during mixing events could be derived from the strong pH contrast between the epilimnion and the lower levels of the lake (~9 and 6 respectively) (Fig. 5). Due to the intrinsic thermodynamic stability properties of aqueous NH$_4^+$, a shift to a higher pH results in the production of significant concentrations of ammonia (NH$_3$) from dissolved NH$_4^+$, which can impose a strong isotope fractionation effect during volatilization, leaving the residual NH$_4^+$ pool $^{15}$N-enriched (Fig. 6)(Li et al., 2012). Initially, the overwhelming volume and H$^+$ ion concentration of the water derived from hypolimnion would minimize this effect during mixing (Fig. 5B). Then, as excess CO$_2$ continued to de-gas and stratification was re-established (Fig. 5C), the pH likely began to increase again, inducing greater rates of volatilization, and NH$_3$ escape to the atmosphere. This effect is almost certainly at play in the modern lake where NH$_4^+$ diffuses across the chemocline across a 2.5 – 3 pH gradient, and during past intervals of similar stratification (Fig. 5A).
However, there is a significant population of NH₃ oxidizers within the lake just above the chemocline, which likely utilize much of the NH₃ and limit its escape to the atmosphere, thus mitigating the expression of any isotope effect in the sediment below (Llirós et al., 2010).

**Figure 3-6:** Effect of pH and NH₃ volatilization on the δ¹⁵N of the residual NH₄⁺ pool constructed using the equations of Li et al. (2012). Inset – Lower mixing event and corresponding stages depicted in Fig. 5. pH effect likely to be more significant during stage C.

### 3.5.3 Pigments:

Sedimentary pigments can be broadly separated into two categories: chlorophylls and carotenoids, inclusive of their derivatives. Carotenoids have a broad structural and functional diversity (Rivera et al., 2014; Roy et al., 2011; Takaichi, 2011) that play important roles in light harnessing (Alberte et al., 1981; Guglielmi et al., 2005; Katoh et al., 1989; Tanada, 1951) as well as photoprotection (Ehling-Schulz et al., 1997; Garcia-Pichel et al., 1992). Carotenoids can also be used as biomarkers in both modern and ancient systems, as many carotenoids are specific to certain classes of organisms (Castañeda and Schouten, 2011; Roy et al., 2011; Takaichi, 2011). In the sediments of Lake Kivu, the carotenoids zeaxanthin and lutein were by far the most abundant pigments in all samples. Lutein in aquatic systems is generally associated with green algae (*Chlorophyta*), while zeaxanthin is predominantly produced by cyanobacteria (Castañeda
and Schouten, 2011; Roy et al., 2011; Takaichi, 2011), though zeaxanthin is present in low abundances in other classes of organisms at high light intensities due to its photoprotective role in the xanthophyll cycle (Goss and Jakob, 2010; Roy et al., 2011). The ratios of zeaxanthin:lutein then can be taken as an estimate of the changing proportion of cyanobacteria:green algal contribution to sedimentary organic matter. In the studied sediments, the zeaxanthin:lutein ratio was generally higher during the mixing events, indicating a higher ratio of cyanobacteria:green algae (Castañeda and Schouten, 2011).

The most common chlorophyll by far across numerous taxonomic classes is chlorophyll $a$, however other chlorophylls and their derivatives with unique structural features can also be used as biomarkers for specific classes of organisms (Keely, 2006; Roy et al., 2011; Scheer, 1991). These structural differences are the result of slightly different biological synthesis pathways, and serve to modify the absorption characteristics of a given chlorophyll in order to absorb different wavelengths of light (Roy et al., 2011; and references therein). For example, specific strains of cyanobacteria that live in visible light-depleted environments beneath other photosynthetic organisms will produce the chlorophylls $d$ and $f$, which have unique structural components that shift the $Q_y$ band of these chlorophylls toward the red/infrared region of the visible light spectrum (Chen et al., 2010; Kühl et al., 2005; Loughlin et al., 2013).

The concentrations of both chlorophylls and carotenoids in a given organism will vary with light intensity, with the magnitude and direction of change depending on the compounds specific function (Ben-Amotz and Avron, 1983; Castenholz and Garcia-Pichel, 2012; Falkowski and Owens, 1980; Garcia-Pichel and Castenholz, 1991; Kana et al., 1988; Latifi et al., 2009; Leisner et al., 1994; Oliveira, 2014; Raps et al., 1983; Schagerl and Müller, 2006). In general, organisms will down-regulate chlorophyll production at high light levels, and up-regulate at low
light intensities (Falkowski and Owens, 1980; Leisner et al., 1994; Paerl, 1984; Schagerl and Müller, 2006). Carotenoids, on the other hand generally increase in concentration under high light intensities, especially pigments such as zeaxanthin which have a specific photoprotective role (Kana et al., 1988; Leisner et al., 1994; Schagerl and Müller, 2006); however some carotenoids such as violaxanthin and fucoxanthin are important in light harvesting, and thus the relationship is not as straightforward as with chlorophylls (Guglielmi et al., 2005; Roy et al., 2011).

Because of the diametric responses of zeaxanthin and chlorophyll a to high light intensities in cyanobacteria (Kana et al., 1988), the ratio of the two compounds can act as a relative irradiance indicator, with higher values indicative of stronger irradiance/light regimes. Because the relative contribution of diatom biomass to TOC in Lake Kivu sediments cannot be constrained due to lack of diatom specific carotenoids, the zeaxanthin:chlorophyllone ratio here can only act as a qualitative metric. Nevertheless, the pattern of higher zeaxanthin:chlorophyllone ratios in the organic-rich sapropels compared to the diatomaceous muds surrounding them (Fig. 3) suggests that cyanobacteria were exposed to a higher light irradiance level during the mixing events. In Lake Kivu today, cyanobacteria overall tend to inhabit a slightly deeper depth habitat than other oxygenic photosynthetic organisms such as diatoms, which mostly live within a few meters of the surface (Sarmento et al., 2012). During mixing stage B when the chemocline is inferred to be closer to the surface, cyanobacteria in the lake may be forced to live closer to the surface, thereby increasing the average light intensity received and inducing higher production of zeaxanthin (Kana et al., 1988; Schagerl and Müller, 2006). Alternatively the changing nutrient regime resulted in an ecological shift towards cyanobacteria that naturally produce more zeaxanthin (Zhang et al., 2018).
On the basis of the UV/VIS absorbance spectra with red shifted Qy band peaks at ~670-674 and 738 nm, we identify the presence of bacteriochlorophyll derivatives, though they are present in abundances too low for detailed identification (Airs and Keely, 2003). Bacteriochlorophyll derivatives are derived from green or purple sulfur/nonsulfur bacteria (Keely et al., 1990; Roy et al., 2011; Scheer, 1991; Wilson et al., 2004). In Lake Kivu, there is no evidence for anoxygenic photosynthesis near the chemocline of the main basin, which precludes the production of high levels of bacteriochlorophylls (Morana et al., 2016). However, there is some evidence for Chlorobium, phototrophic sulfide oxidizing green sulfur bacteria, in Kabuno Bay, a restricted bay connected by a narrow channel at the North end of the basin (Fig. 1) (Morana et al., 2016). The major bacteriochlorophyll producing organisms (green and purple sulfur and non-sulfur bacteria) are restricted to dysoxic/anoxic regions of water columns (Van Gemerden, 1983), and have been known to subsist on extremely low photon-incidence rates (Overmann et al., 1992). In the modern lake, the mixing depth:euphotic depth ratio ($Z_{mix}/Z_{eu}$) is $>1$, suggesting that little light is received at/near the chemocline where free sulfide is present (Sarmento et al., 2012, 2006). The high $Z_{mix}/Z_{eu}$ ratio therefore may explain the lack of anoxygenic photosynthesis in the chemocline of the modern lake, where the high sulfide and nutrient content would otherwise provide an ideal habitat (Fig. 5A) (Van Gemerden, 1983). The presence of bacteriochlorophylls in the high TOC and mixing event sediments implies that the chemocline was shallower during their deposition compared to today, allowing for high enough photon fluence rates within the regions of the water column suitable for green/purple bacterial to grow. As such, the bacteriochlorophyll derivatives described here provide further evidence for the breakdown of stratification and shallowing of the chemocline for a sustained period after mixing events.
3.5.4 A link to OAE productivity:

The chemocline fluctuations described herein provide a mechanistic explanation for the anomalously $^{15}$N-depleted black shales of past OAEs, through the delivery of accumulated NH$_4^+$ to surface waters, as was proposed by Higgins et al. (2012). In a similar fashion, it may also provide a connection to the protracted deposition of high TOC sediments during OAEs (Jenkyns, 2010; Schlanger and Jenkyns, 1976). In order to sustain high levels of productivity in the surface ocean, an adequate supply of nutrient nitrogen and phosphorous along with select trace metals is required. Numerous studies have described the regeneration of phosphorous from sedimentary phases under anoxic conditions (Ingall et al., 1993) and offered this as a mechanism for sustaining productivity under an “anoxia-productivity feedback” (Mort et al., 2007; Van Cappellen and Ingall, 1996, 1994). The periodic mixing of a stratified aquatic basin with anoxic deep waters provides a physical means for the delivery of regenerated phosphorous and nitrogen to the surface where it can be utilized. The presence of periodic oscillations in the redox structure of ancient anoxic basins has already been proposed for the Frasnian-Famennian biotic crisis (Haddad et al., 2018; Lash, 2017; Uveges et al., 2018) and the PETM (Junium et al., 2018a), and the use of high resolution coupled pigment and stable carbon and nitrogen isotope studies may help further elucidate the role of chemocline variability in anoxic basins.
3.6 Conclusions:

Analyses of pigment and stable carbon and nitrogen isotopic signatures of Lake Kivu sediments has revealed a potential mechanism for the anomalously $^{15}$N-depleted isotopic compositions of past OAEs. Coupled depletions in $\delta^{13}$C$_{org}$ and $\delta^{15}$N$_{bulk}$ values indicate the greater influence of isotopically depleted DIC and NH$_4^+$ derived from below the chemocline during periods of lake mixing events. As well, pigment biomarkers and ratios record a shallowing of the euphotic zone, and shift in the locus of production by cyanobacteria, and provide an additional metric for characterizing past chemocline destabilizations. Future high-resolution pigment and $\delta^{13}$C$_{org}$ and $\delta^{15}$N$_{bulk}$ studies may reveal the impact of chemocline fluctuations on past deoxygenation events/OAEs and organic matter-rich sediment deposition.

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CHAPTER 4: BIOGEOCHEMICAL CONTROLS ON BLACK SHALE DEPOSITION DURING THE FRASNIAN-FAMENNIAN BIOTIC CRISIS IN THE ILLINOIS AND APPALACHIAN BASINS, USA, INFERRED FROM STABLE ISOTOPES OF NITROGEN AND CARBON

Abstract

The Frasnian-Famennian biotic crisis is marked by two distinct intervals known as the Lower and Upper Kellwasser Events (KWEs) that in many locations are associated with deposition of organic-rich shales. Sedimentary nitrogen and carbon isotopes offer insight into the biogeochemical processing of nutrients, production of organic matter, and palaeoceanographic conditions during the KWEs. Here we present new bulk nitrogen ($\delta^{15}N_{\text{bulk}}$) and organic carbon ($\delta^{13}C_{\text{org}}$) isotope data from the Late Devonian Appalachian and Illinois Basins (AB and IB), with a focus on intervals encompassing the KWEs. Black shales from the IB and AB, including the KWEs, are $^{15}N$-depleted ($-1.0 - +2.0 \%$o) and have significantly lower $\delta^{15}N_{\text{bulk}}$ than interbedded grey shales ($+0.5 - +4.0 \%$o), a trend consistent with many instances of black shale deposition in the Phanerozoic. Organic carbon isotopes exhibit the broad, positive excursions ($\sim +3.5 \%$o from background) that are typical of the KWEs globally. Superimposed over these positive excursions in $\delta^{13}C_{\text{org}}$ are sharp decreases of up to $\sim3.0 \%$o within the black shale beds, to as low as $-30.5 \%$o.

The pattern of $\delta^{15}N_{\text{bulk}}$ and $\delta^{13}C_{\text{org}}$ values suggests that the depth of the chemocline and the degree of water-column stratification exert a primary control on both $\delta^{15}N_{\text{bulk}}$ and $\delta^{13}C_{\text{org}}$ during black shale deposition. In the context of the Frasnian-Famennian biotic crisis, the oscillating redox state and changing temperatures would have likely placed extreme stress on organisms within the marine environment of the AB and IB and may potentially have been a contributing factor to diversity loss over this time period.
4.1 Introduction

The Late Devonian was a period characterized by intense climatic and ecological change, highlighted by the expansion of land plants (Algeo and Scheckler, 1998; Knoll et al., 1984), a dramatic drop in atmospheric CO_2 levels (Royer, 2006; Simon et al., 2007; Xu et al., 2012), intense and widespread tectonic/volcanic activity (Averbuch et al., 2005; Racki, 1998; Racki et al., 2018; Ricci et al., 2013), and the Frasnian-Famennian biotic crisis. The Frasnian-Famennian biotic crisis ranks among the top five mass extinctions in ecological severity (McGhee et al., 2013; Sepkoski, 1996), was particularly devastating to shallow-water tropical faunas and reef systems (Bambach et al., 2004; Bambach, 2006; Copper, 2002; McGhee, 1996; Racki, 2005), and was unique in that it exhibits several pulses of elevated extinction rates coupled with depressed origination rates (Bambach, 2006; McGhee, 1996; Stigall, 2012). The roles of rapid environmental change and the widespread deposition of organic-matter-rich shales in the Frasnian-Famennian biotic crisis remain items of intense interest, as the root cause(s) of the biotic crisis remain thus far unresolved (Racki, 2005).

What is clear is that the Frasnian-Famennian biotic crisis is globally correlated with the deposition of a set of beds known as the Kellwasser intervals named for bituminous horizons initially described from the Kellwasser limestone of Germany (Becker, 1986; Roemer, 1850). Well defined by conodont biostratigraphy (Day and Witzke, 2017; Girard et al., 2005; Klapper and Feist, 1993; Over, 2002), the KWEs are identified in Europe, the United States, Canada, North Africa, China, and Australia (Becker et al., 1991; Becker and House, 1997; Bond et al., 2013; Casier, 1987; Chen et al., 2005; Crick et al., 2002; Day and Witzke, 2017; Feist, 2002; George et al., 2014; House, 2000; Lash, 2017; Levman and Von Bitter, 2002; Over, 2002; Pujol
et al., 2006; Racki et al., 2002; Riquier et al., 2006; Wednt and Belka, 1991; Whalen et al., 2002; Xu et al., 2012). Many of these sections show significant total organic carbon (TOC) enrichment, as high as 20 wt.%, within the KWEs, though this is not true of all sites. However, all KWE sites record, to some degree, prominent increases in the $\delta^{13}C$ of carbonate and organic carbon (Hillbun et al., 2015), which are consistent with sequestration of $^{13}C$-depleted carbon (cf., Joachimski and Buggisch, 1993; Kump and Arthur, 1999), presumably as organic carbon in sediments.

The presence of anoxic/dysoxic and/or euxinic conditions at many locations across the Frasnian-Famennian interval is well-documented on the basis of trace metal geochemistry (Algeo, 2004; Averbuch et al., 2005; Bond et al., 2013; Boyer et al., 2014; Joachimski et al., 2001; Long et al., 2015; Over, 2002; Rimmer, 2004; Riquier et al., 2006; Sageman et al., 2003), biomarker evidence (Brown and Kenig, 2004; Haddad et al., 2016; Joachimski et al., 2001), and ichnofabric studies (Boyer et al., 2014; Haddad et al., 2018). Although anoxia is a central theme in paleoenvironmental studies that consider the deposition of Kellwasser black shales, there is no clear consensus on the forcing mechanism(s). Some authors stress marine transgression allowing anoxic deep waters to spill over into epicontinental seas and shelf basins (Bond et al., 2004; Bond and Wignall, 2008; Day and Witzke, 2017; Johnson et al., 1985), while others argue for regression-induced oceanic overturn (Chen et al., 2013; Levman and Von Bitter, 2002; Sandberg and Ziegler, 1988), and still others propose episodic shallow water eutrophication initiated by myriad mechanisms, including increased nutrient regeneration within a water column experiencing redox oscillation, or increased nutrient runoff (Carmichael et al., 2014; Huang and Gong, 2014; Kazmierczak et al., 2012; Murphy et al., 2000a). In order to better understand conditions associated with the biotic transition/extinction during this time, we present nitrogen and organic carbon isotope data that span the KWEs from the Appalachian Basin(AB) of
Western New York State and the Illinois Basin (IB) of eastern Iowa, that lend insight into the nutrient cycling and palaeoceanographic dynamics of these basins.

Carbon isotopes of marine organic matter ($\delta^{13}$C$_{org}$) can serve as a tracer for the global carbon cycle (cf. Kump and Arthur, 1999) as well as provide insight into local carbon cycling dynamics, sources of organic matter, and microbial ecology. Nitrogen isotopes ($\delta^{15}$N) serve as a proxy for nutrient dynamics, sources of biologically available nitrogen, and the degree to which redox processes affect the isotopic composition of reactive nitrogen reservoirs. Specifically, $\delta^{13}$C$_{org}$ and $\delta^{15}$N$_{bulk}$ data help to illuminate the interplay of regional water-column redox structure and primary producer communities in the AB and IB and can help provide perspective on the biotic crisis as a whole. Based upon our results, we propose that primary production was fueled by $^{13}$C-depleted dissolved inorganic carbon and $^{15}$N-depleted ammonium derived from the mineralization of organic matter below the chemocline. These nutrients were then either utilized by chemocline-dwelling autotrophs, or mixed into the overlying photic zone, accounting for the coupled $\delta^{13}$C$_{org}$ and $\delta^{15}$N$_{bulk}$ depletion in KWE interval black shales of the sections studied here.

4.2 Geologic setting

4.2.1 Appalachian Basin

During the Late Devonian, much of the eastern United States was under a broad epeiric seaway that included the Appalachian, Illinois and Michigan Basins. It should be noted here that North America has undergone a $\sim$45° rotation since the Late Devonian. For clarity, all geographic relationships described are done so in relation to the modern position of the given geographic feature, which is represented in the upper insert of Fig. 1. The AB is a foreland basin that formed as a result of crustal loading during the Acadian Orogeny. It extends from New York
State in the north, southeast to Alabama, south to Mississippi, and west to the Cincinnati Arch in central Ohio (Ettensohn, 1985a; Faill, 1985) (Fig. 1.). The orogenic belt to the east provided siliciclastic sediment that filled the subsiding foreland basin resulting in an eastward-thickening, westward-prograding clastic wedge (Murphy et al., 2000). The basin comprises proximal deltaic deposits in the east that grade into distal, fine-grained siliciclastics to the west towards the Cincinnati-Findlay-Kankakee Arch complex (Ettensohn, 1985b; Sageman et al., 2003; Woodrow et al., 1988). The degree of deep water connection across the Cincinnati Arch is thought to have played an important role in the ventilation and water-column dynamics of the AB and is central to the processes that are thought to have fostered the deposition of black shale facies (Algeo et al., 2007; Ettensohn and Elam, 1985). Periods of relative sea level change or eustasy modulated the deep-water connection between the AB and the greater North American Epeiric Sea to the south and east.

The AB section studied here is exposed along Walnut Creek, located near the town of Silver Creek in Chautauqua County of western New York State, and is described in detail by Lash (2017). Overall, the site consists of distal deltaic deposits, that accumulated in relatively shallow water (Ettensohn, 1985b; Murphy et al., 2000b) at least 250 km from shore (Brett and Baird, 1996; Murphy et al., 2000b). The base of the section is the upper portion of the Angola Formation, which is comprised of organic matter lean, silty grey shales. Immediately overlying the Angola Formation is the Pipe Creek formation, a ~65 cm thick organic-rich black shale that, on the basis of conodont and carbon isotope stratigraphy, corresponds with the Lower KWE (Over, 2002). Above the Pipe Creek, ~29 m of the Hanover Formation is exposed. The Hanover is composed principally of silty grey shale, intercalated with numerous thin (5-10 cm) black shale beds and carbonate concretion layers (Lash, 2017, 2015). The Upper Kellwasser Event is
represented by a ~18 cm thick black shale that lies 80 cm below the Hanover-Dunkirk contact (Over, 2002). The relatively shallow and large AB was likely somewhat heterogeneous in its biogeochemical structure. However, our data are consistent with those from other sites in the AB (Sageman et al., 2003; Tuite and Macko, 2013), and so we use the acronym AB throughout this study to compare our Walnut Creek site to those in the IB.

**Figure 4-1:** Paleogeographic reconstruction of continents, and North America (insert) during Frasnian-Famennian biotic crisis. Circles represent known sample sites with TOC data. Left half of circle represents LKE TOC, and right half of circle the UKE. White stars on inserts represent this studies sampling locations. Maps modified after Blakey, 2005 and Algeo et al., 2007. Additional TOC data from Chen et al., 2005; Formolo et al., 2014; George et al., 2014; Haddad et al., 2016; Joachimski and Ostertag-Henning, 2001; Lash, 2017; Levman and Von Bitter, 2002; Pujol et al., 2006; Xu et al., 2012.
4.2.2 Illinois Basin

The IB is a sag basin centered in Illinois, western Indiana, and western Kentucky (Cluff, 1980; de la Rue et al., 2007). It is enclosed to the south and east by the Cincinnati Arch, the Kankakee and Wisconsin highs to the north, and the Ozark Uplift to the south and west (Cluff, 1980; Over, 2002; Schieber and Lazar, 2004; Willman et al., 1975). However, the basin maintained a marine connection to the Ouchita continental margin and Rheic Ocean between the Cincinnati Arch and Ozark Uplift through southern Illinois (Fig. 1). The presence of the Cincinnati Arch likely acted to restrict sediment transport from the AB, which may account for the relative thinness of IB deposits compared to equivalent strata of the AB (Cluff, 1980). Like the AB, the IB was probably no deeper than a few hundred meters (Algeo and Maynard, 1997; Jaminski et al., 1997; Potter et al., 1982).

The LKE of the western IB is hosted by an interval of thermally pristine (conodont CAI of 1) medium grey-green calcareous shales; the UKE interval is found within organic-rich brown shales (Day and Witzke, 2017). The studied IB sections include the type section of the Late Frasnian Sweetland Creek Shale where it is overlain by the latest Frasnian and Early Famennian Grassy Creek Shale in Muscatine County, Iowa (site referred to as TSC), and the highly condensed Sweetland Creek and Grassy Creek section in the Iowa Geological Survey (IGS) Sullivan Core from the Sullivan Slough Quarry just south of the city of Burlington, Iowa (Day and Witzke, 2017). Both sites, located on the western margin of the IB, display deep ramp facies of the epeiric Lime Creek Formation carbonate platform. The LKE and UKE intervals and Frasnian-Famennian boundary at TSC are well constrained by conodont biostratigraphy (see Day and Witzke, 2017). The offshore conodont sequence at the TSC site (Day and Witzke, 2017)
serves as the principal reference section for the Late Frasnian, Frasnian-Famennian Boundary and Early Famennian interval in central North America (Johnson and Klapper, 1992).

The TSC section studied here is ~3.9 m thick and is comprised of the upper 2.7 m of the Sweetland Creek shale, and the lower 1.2 m of the Grassy Creek shale (Fig. 2). This section is part of a larger section described in detail by Day and Witzke (2017). The LKE interval is 30 cm thick within the lower part of a 106 cm-thick grey/green shale unit. Its base is located 17 cm above a green siltstone and its upper boundary is marked by the base of Frasnian Subzone 13a (Fig. 2) (Day and Witzke, 2017). The UKE comprises 56 cm of organic-rich brown shale which contains several thin grey shales, and volcanic ash layers in the lower Grassy Creek Shale (Fig. 2) (Day and Witzke, 2017).

The Sweetland Creek Shale of the IGS Sullivan core embodies 85 cm of thin dolomite, limestone, and calcareous mudstone that displays closely spaced (centimeter scale) stacked hardgrounds in both the lower and upper parts of the unit (Day and Witzke, 2017). The upper contact of the ~20 cm thick LKE corresponds with the contact of the Sweetland Creek and Grassy Creek shales (Fig. 2). The upper boundary of the ~20 cm thick UKE is found within the lower Grassy Creek shale (Fig. 2) at the base of a discontinuity described by Day and Witzke (2017). As with the AB, the IB likely was somewhat heterogeneous in its biogeochemical structure. Again however, our data are consistent with data from northern Indiana (de la Rue et al., 2014), and therefore we use the IB acronym to compare our sites to the AB.
4.3 Methods:

4.3.1 Sample collection

Samples of black and grey shales of the Hanover and Pipe Creek members of the Java Formation from western New York were obtained from outcrops exposed along the bed and banks of Walnut Creek, using the boundary definitions of Over (2002). Samples were collected at ~10 cm spacing within gray shale layers and at intervals of 1 to 3 cm within the black shale layers. Brown and grey shales from the type section of the Sweetland Creek Shale were sampled at 10 cm intervals. The Sweetland Creek in the IGS Sullivan core was sampled at 2 to 3 cm intervals and the Grassy Creek at 5 to 10 cm intervals (Day and Witzke, 2017). Samples were physically cleaned of exogenous debris, washed and sonicated in 18.2 MΩ deionized water, and rinsed with UHPLC grade Methanol. Once dry, samples were powdered using a tungsten carbide ball-mill, or a steel mortar and pestle. Powders were acidified with 3 N hydrochloric acid, rinsed with deionized water and freeze-dried prior to analysis. The %C reported here is determined for the residual sample after acidification. 84 samples from ~5 m of section at the Walnut Creek site, 40 samples from ~4 m of section at the TSC site, and 14 samples across ~1 m from the IGS-Sullivan core were analyzed.

4.3.2 Bulk stable isotope analyses

Nitrogen and carbon isotopic analyses of decarbonated rock powders were performed in the Syracuse University GAPP Lab using an Elementar Isotope Cube elemental analyzer (EA) coupled directly to an Isoprime 100 isotope ratio mass spectrometer (IRMS) using conventional techniques for EA-IRMS. Rock powders were weighed into tin cups, evacuated, and sparged with Ar to remove interstitial N₂. EA conditions were as follows: helium purge was set for 30
seconds, oxidation and reduction reactor temperatures were 1100 °C and 650 °C, respectively; helium carrier gas flow was 230 ml/min; and the O₂ pulse was set for 60 seconds. International reference materials IAEA N1 ammonium sulfate [δ¹⁵N= +0.4 ‰]; N2 ammonium sulfate [δ¹⁵N = +20.3 ‰]; NIST 1547 peach leaves [δ¹⁵N = +2.0 ‰; δ¹³C = −26.0 ‰]; ANU Sucrose [δ¹³C = −10.45 ‰] and Messel Oil Shale (in house standard [δ¹⁵N = +7.0 ‰]) were used in concert with sample material for reference calibrations. The resulting blank corrected sample and standard data were corrected to accepted values for the reference materials using the correction scheme described in Coplen et al., (2006). Reproducibility for samples and standards for δ¹⁵N was better than +/- 0.2 ‰ but is reported as such to reflect the reported nitrogen isotopic composition of the reference materials (+/- 0.2 ‰). Reproducibility for samples and standards for δ¹³C was +/- 0.1 ‰. Carbon isotope values are presented relative to Vienna Peedee Belemnite (VPDB), and nitrogen isotope values relative to atmospheric N₂.

4.4 Results:

4.4.1 Carbon

δ¹³C₉ₒᵣg values ranged from −30.5 to −26.0 ‰ across both basins, and displayed the broad, positive excursions typical of the KWEs globally (Fig. 2). Superimposed on these excursions in δ¹³C₉ₒᵣg are sharp decreases, to as low as −30.5 ‰, located at the base of the black/brown shale beds, congruent with the data of Lash (2017). Overall, there was no significant correlation between δ¹³C₉ₒᵣg and %C. However, at the Walnut Creek section (the most expanded section investigated), black shales were ¹³C-depleted by an average of 1.6 ‰ relative to the immediately adjacent interbedded grey shale, with the lowest δ¹³C₉ₒᵣg occurring at the base of the Kellwasser black shales (Fig. 2, and Table 1). This relationship is also observed in the TSC section, where
δ^{13}C_{\text{org}} of brown shale averages 1.2 % less than the adjacent grey shale (see Fig. 2 and Table 1 for further method description). This relationship was not apparent in the IGS-Sullivan sample set, likely due to the compressed nature of the IGS-Sullivan site (KWEs encompassed by ~4 m at TSC vs ~1 m at IGS-Sullivan) and the lithology of available samples which are primarily brown organic-rich shales. Again, there was no significant relationship between δ^{13}C_{\text{org}} and %C over the total section investigated in either basin (TSC r = −0.16, p > 0.05, n = 39 [this study]; IGS-Sullivan r = −0.36, p > 0.05, n = 14 [this study]; Walnut Creek total section determined by Lash, 2017; r = -0.02 (no p or n values reported)), and the general pattern of^{13}C-depletion in black/brown shales was observed only when comparing immediately adjacent shale beds (see Table 1).

**Figure 4-2:** Carbon (black circles) and Nitrogen (black triangles) isotope and %C of acidified sample residuals (grey diamonds) profiles of Walnut Creek, TSC and IGS-Sullivan core samples. Trend contained inside the dark grey box within the Pipe Creek shale of the Walnut Creek section are based on the data of Lash (2017). KWEs are represented by the light grey rectangles across the profiles.
The %C of acidified sample residuals range from 0.2 - 10.2 %, and 0.1 % - 5.7 %, and 
$C_{org}/N$ ratios range from 1.8 - 32.9 and 1.4 - 27.7 in the AB (Walnut Creek site) and IB (TSC and 
IGS-Sullivan sites), respectively, with the highest %C values occurring within KWE 
brown/black shale of both basins. The LKE of the Walnut Creek section displays the highest %C 
(10.2 %). $C_{org}/N$ values are elevated in the black/brown shales, which is typical of Phanerozoic 
black shales (Junium and Arthur, 2007), with the highest values again occurring within the LKE 
bed of the Walnut Creek section (32.9) (see Fig. 2 and Table 1 in supplement).

**Table 4-1**: Black/brown and grey shale $\delta^{13}C_{org}$ offsets

<table>
<thead>
<tr>
<th>WC Data (beds grouped by lithology) $^a$, $^b$</th>
<th>Avg. $\delta^{13}C_{org}$ (%) $^c$</th>
<th>Avg. Difference $^d$, $^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed 1 (–88 - –2 cm)</td>
<td>−28.8</td>
<td></td>
</tr>
<tr>
<td>Bed 2 (LKE) (0 – 65 cm)</td>
<td>−29.5 $^g$</td>
<td>−1.9</td>
</tr>
<tr>
<td>Bed 3 (76 – 141 cm)</td>
<td>−26.4</td>
<td></td>
</tr>
<tr>
<td>Bed 4 (142 – 149 cm)</td>
<td>−27.6</td>
<td>−1.2</td>
</tr>
<tr>
<td>Bed 5 (161 – 191 cm)</td>
<td>−26.4</td>
<td></td>
</tr>
<tr>
<td>Bed 6 (216 cm)</td>
<td>−28.0</td>
<td>−1.5</td>
</tr>
<tr>
<td>Bed 7 (246 – 276 cm)</td>
<td>−26.5</td>
<td></td>
</tr>
<tr>
<td>Bed 8 (303 cm)</td>
<td>−28.2</td>
<td>−1.5</td>
</tr>
<tr>
<td>Bed 9 (323 – 346 cm)</td>
<td>−26.8</td>
<td></td>
</tr>
<tr>
<td>Bed 10 (370 cm)</td>
<td>−28.7</td>
<td>−1.9</td>
</tr>
<tr>
<td>Did Not Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bed 11 (–55 - –2 cm)</td>
<td>−28.7</td>
<td></td>
</tr>
<tr>
<td>Bed 13 (UKE) (0.5 – 17 cm)</td>
<td>−29.9</td>
<td>−2.0</td>
</tr>
<tr>
<td>Bed 12 (21 – 30 cm)</td>
<td>−27.1</td>
<td></td>
</tr>
<tr>
<td>Bed 13 (34 cm)</td>
<td>−28.1</td>
<td>−1.2</td>
</tr>
<tr>
<td>Bed 14 (44 cm)</td>
<td>−26.5</td>
<td></td>
</tr>
<tr>
<td>Bed 15 (55 – 80 cm)</td>
<td>−28.6</td>
<td>−1.4</td>
</tr>
<tr>
<td>Bed 16 (81 – 92 cm)</td>
<td>−27.9</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>−1.6</td>
</tr>
<tr>
<td>Avg. (%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1: Continued

<table>
<thead>
<tr>
<th>TSC Data (beds grouped by lithology)</th>
<th>Avg. δ\textsuperscript{13}C\textsubscript{org} (%)</th>
<th>Avg. Difference\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>a, b Bed 1 (samples 12 - 16)</td>
<td>−28.4</td>
<td></td>
</tr>
<tr>
<td>Bed 2 (samples 17 - 20)</td>
<td>−29.7</td>
<td>−1.4</td>
</tr>
<tr>
<td>Bed 3 (samples 21 - 25)</td>
<td>−28.1</td>
<td></td>
</tr>
<tr>
<td>Bed 4 (sample 26)</td>
<td>−30.1</td>
<td>−1.7</td>
</tr>
<tr>
<td>Bed 5 (sample 27)</td>
<td>−28.6</td>
<td></td>
</tr>
<tr>
<td>Bed 6 (samples 28 - 32)</td>
<td>−28.9</td>
<td>−0.3</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>−1.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a} ‘Beds’ defined by sample position/range shown in parentheses (see Table 1 in the supplement)
\textsuperscript{b} Black/brown shales are shaded, grey shales have no shading
\textsuperscript{c} Bed average δ\textsuperscript{13}C\textsubscript{org} (%) data
\textsuperscript{d} Average differences between black shale beds and immediately adjacent grey shale beds (see Eq. 1. Bed\textsubscript{i} is always a black/brown shale). Eq. 1: Column 3 = \frac{((\text{Bed}\textsubscript{i})−\text{Bed}\textsubscript{i−1})+(\text{Bed}\textsubscript{i})−\text{Bed}\textsubscript{i+1}}{2}
\textsuperscript{e} Bottom right most cell is the average difference for the section. Due to the compressed nature of the TSC section (4 m at TSC vs ~30 m at WC for duration of the KWEs and intervening period) the largest beds, specifically beds 1 and 6, were restricted to 5 samples when determining their average. This was done to mitigate the influence of the long-term global signal and accentuate the finer scale local short-term signals imposed by chemocline production.
\textsuperscript{g} Value calculated including data from Lash (2017). See Table S1 in the supplement for further description.

4.4.2 Nitrogen

Nitrogen isotopic composition of Walnut Creek samples ranges from −1.0 to +1.8 ‰, with black shales being \textsuperscript{15}N-depleted by an average of −1.1 ‰ compared to the grey shales. The range of values in the black shales is similar to that reported by Tuite and Macko, (2013) for the UKE intervals of Walnut Creek and two other AB sites. The two KWE intervals exhibited the lowest δ\textsuperscript{15}N\textsubscript{bulk}, with LKE and UKE samples ranging from −1.0 to −0.1 ‰, and averaging −0.5 and −0.6 ‰ respectively (combined average = −0.5 ‰; stdev. = 0.4 ‰). The KWEs on average
were depleted by ~0.8 and 1.6 ‰ compared to other black (avg. = +0.3 ‰; stdev. = 0.3 ‰) and
grey shale beds (avg. = +1.1 ‰; stdev. = 0.3 ‰) respectively, of the Walnut Creek section.
Overall, δ¹⁵N values define three main distributions (see Fig. 3, and supplement Table S2 for KS
statistics) on the basis of %C, with the KWEs having the lowest δ¹⁵N, and highest %C (Fig. 3).
δ¹⁵N values of the Sweetland Creek type section (TSC) range from 0.0 to +4.0 ‰, with the
brown shale (avg. = +1.0 ‰) more depleted by ~2.3 ‰ compared to the grey shale (avg. = +3.3
‰). Nitrogen isotope values of the IGS-Sullivan core sample suite range from +0.5 ‰ to +2.7 ‰ and
display the same pattern with grey shale averaging +2.6 ‰ and brown shale averaging +1.2
‰. Nitrogen isotope values of gray and brown shale of the IB sections averaged +3.3 ‰ (stdev.
= 0.4 ‰) and +1.1 ‰ (stdev. = 0.5 ‰) respectively. The pattern of brown shale depletion and
overall δ¹⁵N values are consistent with those reported by de la Rue et al., (2007) from an IB
section in Indiana to the southeast of those studied here. In general, δ¹⁵N and %C display strong
covariance (Walnut Creek: r = −0.79, p << 0.01, n = 78; TSC: r = −0.93, p << 0.01, n = 39; IGS-
Sullivan: r = −0.84, p << 0.01, n = 14) with higher %C values corresponding with lower δ¹⁵N
values. These correlations were only apparent when considering the full data set, as the
individual shale groups showed no significant correlations. The δ¹⁵N of all lithologies studied
here were normally distributed (see supplementary Fig. S1 for Shapiro Wilk p values, and Q-Q
plots).
Figure 4-3: Histograms of $\delta^{15}\text{N}_{\text{bulk}}$ data: (A) Walnut Creek section of the Appalachian Basin with 0.25 ‰ bin widths (this study); (B) TSC and IGS Sullivan sites of the Illinois Basin with 0.5 ‰ bin widths (this study); (C) Mediterranean Sea site 969 data of Milder et al., (1999) with 1.0 ‰ bin widths; (D) Compiled data from all sites with 0.5 ‰ bin widths. Black and grey dots represent the average and standard deviation of a given lithology, dashed curves show normal distributions. Each distribution is distinct from the other distributions at a given site (see supplementary data Table S2 for KS test statistics).
4.5 Discussion

4.5.1 Carbon isotopes

The carbon isotopic composition of organic matter at any given location is dependent on a number of factors, including $pCO_2$ (Freeman and Hayes, 1992; Hollander and McKenzie, 1991), microbial ecology, the biosynthetic pathway of carbon fixation (House et al., 2003; Hügler and Sievert, 2011), the $\delta^{13}C$ of dissolved inorganic carbon (DIC) being utilized for organic carbon production (Fogel and Cifuentes, 1993), and the extent of heterotrophy. During global carbon cycle perturbations, $\delta^{13}C_{org}$ typically mirrors $\delta^{13}C_{carb}$. However, modulation of the factors noted above on a local scale over short time periods can decouple local $\delta^{13}C_{org}$ from the exogenic carbon cycle leading to the preservation of local signals superimposed over the global carbon cycle $\delta^{13}C_{org}$ record.

The Upper and Lower KWEs of the Frasnian-Famennian biotic crisis exhibit globally correlated, positive $\delta^{13}C$ excursions on the order of +2.0 to +3.0 ‰ in both organic and carbonate carbon phases (Joachimski and Pancost, 2002; Hillbun et al., 2015) consistent with an increase in the fractional burial of $^{13}C$-depleted carbon (Joachimski and Buggisch, 1993; Kump and Arthur, 1999) (Fig. 1). It is noteworthy, however, that total organic carbon (TOC) enrichment is not characteristic of all locations that express the $\delta^{13}C$ excursion. For example, the Kellwasser intervals of the Canning Basin of Australia are characterized by a positive $\delta^{13}C_{carb}$ excursion though TOC remains low throughout (George et al., 2014). Such examples highlight the global impact of this event on the carbon cycle.

An additional curious aspect of the Kellwasser record is the variable phasing of the $\delta^{13}C$ excursion relative to the TOC maxima (Hillbun et al., 2015) and the Frasnian-Famennian boundary, as defined by the base of the *Palmatolepis triangularis* conodont zone (House, 2000).
Sites with inferred Frasnian-Famennian boundaries where organic matter-enriched strata are present can display peak δ^{13}C excursions that occur within or above the organic-rich deposits associated with the event (Hillbun et al., 2015). Flexibility in the phasing of δ^{13}C excursions and TOC enrichment are well described from the strata of the Cenomanian-Turonian Oceanic Anoxic Event 2 (OAE 2) (Tsikos et al., 2004). Like OAE 2, the specific stratigraphic trend of δ^{13}C and TOC at any one site during the Frasnian-Famennian biotic crisis was dependent on a suite of local variables that were superimposed on the global carbon cycle signals.

The δ^{13}C of particulate organic carbon in the anoxic region of stratified basins can be significantly lower in the chemocline than that of organic carbon produced in surface waters (Havig et al., 2017; Velinsky and Fogel, 1999). This signal is the result of the combined effects of remineralization of δ^{13}C-depleted organic matter at depth, differing microbial ecologies and pathways of carbon fixation, and the oxidation of the byproducts of fermentation, such as methane. In modern anoxic basins such as Framvaren Fjord and Fayetteville Green Lake, δ^{13}C-depleted organic carbon produced within the chemocline can depress δ^{13}C_{org} in the underlying sediments (Havig et al., 2017; Velinsky and Fogel, 1999).

The δ^{13}C_{org} values within the black/brown shales of the KWEs at all the studied sites mirror the global δ^{13}C increase observed in carbonates and the organic carbon records of other sites (Fig. 4) but display negative offsets of up to 3.0 ‰, with an average offset of ~2 ‰ (Figs. 2 and 4, Table 1) (Lash, 2017; This study). This offset is also reported in the Pipe Creek shale of central New York by Sageman et al., (2003). Discrete black shale layers not associated with the KWEs also show a negative offset from adjacent grey shale. Facies dependent offsets in δ^{13}C_{org} is a common feature of the Middle to Late Devonian strata of western New York and is not limited to the Frasnian-Famennian interval (Sageman et al., 2003). It should be noted here that
the $\delta^{13}\text{C}_\text{org}$ dependence described here is only observed when comparing immediately adjacent shale layers at high resolution, as the sections studied here display no broad correlation between $\delta^{13}\text{C}_\text{org}$ and $\%\text{C}$ (Lash, 2017; Sageman et al., 2003; This study) indicating that preferential degradation and $^{13}\text{C}$-enrichment of organic matter under oxic conditions had a minimal effect on $\delta^{13}\text{C}_\text{org}$.

**Figure 4-4**: Idealized illustration of global carbonate $\delta^{13}\text{C}$ signal (first panel), and localized $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signals. Phosphate $\delta^{18}\text{O}$ data is from conodont apatite (Joachimski and Buggisch, 2002; Joachimski et al., 2004). Negative deviations from background in $\delta^{18}\text{O}$ indicate warming, while positive deviations indicate cooling. $\delta^{15}\text{N}$ values shown here are based on WC section, IB sections show similar pattern, but range from 0-4 ‰.

An influx of terrestrial detrital organic matter as a source of light carbon is a reasonable concern, especially in the relatively enclosed AB. However, macerates from Walnut Creek reveal organic carbon that is wholly of marine origin, on the basis of a lack of terrestrial palynomorphs/plant material (see supplement Fig. S2). Furthermore, biomarker data are dominated by marine sources of organic matter (Haddad et al., 2016). Additionally, different fractions of organic matter have been shown to have differing average $\delta^{13}\text{C}$ values compared to bulk organic matter, with carbohydrates and proteins being generally $^{13}\text{C}$-enriched and lipids
being $^{13}$C-depleted compared to bulk biomass (Degens, 1969; Deines, 1980). Carbohydrates and proteins are more susceptible to microbial degradation (Harvey et al., 1995; Hedges et al., 1988) and thus the preferential preservation of $^{13}$C-depleted lipids could result in negative shifts in sedimentary $\delta^{13}$C$_{\text{org}}$. However, degradation studies have shown that the overall effect of this preferential preservation is essentially equal under anoxic and oxic shallow diagenetic conditions (Lehmann et al., 2002), and therefore this mode of diagenesis is unlikely to be the root cause of the observed offsets between black/brown and grey shales in the AB and IB.

Considering the mechanisms of similar carbon cycle signals in modern anoxic basins, we suggest that the ~2‰ average offset in $\delta^{13}$C$_{\text{org}}$ in the IB and AB KWE black/brown shales is the result of chemocline-derived production where microbial communities with varying pathways of carbon fixation (House et al., 2003; Hügler and Sievert, 2011) assimilated $^{13}$C-depleted DIC (e.g. Fogel and Cifuentes, 1993; Havig et al., 2017) or organic compounds such as methane (e.g. Lash, 2017). However, simple variation of the proportion of methanotroph biomass was unlikely to have exerted a dominant control on $\delta^{13}$C$_{\text{org}}$ variability. Despite significant variability in $\delta^{13}$C$_{\text{org}}$ at Walnut Creek during deposition of the UKE (Fig. 2), 3β-methylhopane indices were consistent and well within Phanerozoic averages (Haddad et al., 2016). While it is clear methanotrophic activity was important, production by other chemocline-dwelling autotrophs and/or mixing of $^{13}$C-depleted DIC into the photic zone during water-column mixing could yield similar low $\delta^{13}$C$_{\text{org}}$ values in a manner similar to that proposed for Toarcian black shales (cf. Küspert, 1982). This mode of carbon cycling appears to have characterized black shale deposition during the KWE the AB and IB and more broadly within the Appalachian Basin over much of the Late Devonian (Sageman et al., 2003), and thus was likely also a contributing factor to the observed offset. It should be noted that when referring to the AB as “enclosed or
restricted” we do not mean to suggest that it was not in some contact with the global ocean (at the very least through surface waters). Indeed, the fact that these sections do display the large positive $\delta^{13}$C excursions typical of the KWEs globally is proof of the connection to the global carbon reservoir (Fig. 4). We refer to the basin as restricted in the sense that it was inferred to be mostly enclosed to the north and west, and partially to the south, as well as at depth by the Cincinnati-Findlay-Kankakee Arch complex, and a sill to the south (Algeo et al., 2007). This likely helped slow/limit deep-water exchange, as well as increase the amount of freshwater runoff delivered to the surface of the basin.

4.5.2 Nitrogen

4.5.2.1 The nitrogen cycle and $\delta^{15}$N

Marine primary productivity is principally controlled by concentrations of the primary limiting nutrients phosphate and dissolved inorganic nitrogen (DIN), as well as trace elements, including iron and molybdenum. The marine nitrogen cycle is modulated by the input of DIN by diazotrophic (N$_2$-fixing) bacteria and the loss of DIN that occurs under reducing conditions. Nitrate reduction (denitrification), and anaerobic ammonium oxidation (anammox), which occur in anoxic water columns and sediments (Brandes et al., 2007; Canfield et al., 2010), are the principal sinks for DIN in the ocean (e.g. Lam et al., 2009; Ward et al., 2009). Rates of diazotrophy and denitrification/anammox show a degree of spatial coupling as the loss of DIN acts to decrease the N:P ratio in the water column, creating ecologically advantageous conditions for phosphorus dependent diazotrophs once DIN has been depleted (Deutsch et al., 2007). However, a phosphorus surplus is not necessarily dependent on denitrifier activity, as phosphorus is largely supplied by weathering (Benitez-Nelson, 2000) and is efficiently
regenerated from sedimentary phases under anoxic conditions that can further depress N:P ratios (Adams et al., 2010; Ingall and Jahnke, 1997; Ingall et al., 1993). Though most phytoplankton communities display flexibility in their elemental stoichiometry and ecologies under variable nutrient conditions (Falkowski, 1997; Galbraith and Martiny, 2015; Hillebrand and Sommer, 1999; Redfield, 1958), N:P ratios and the redox state largely regulate the nitrogen cycle (Loladze and Elser, 2011; Quan and Falkowski, 2009).

The aforementioned nitrogen cycle processes are accompanied by measurable stable isotope fractionations (ε) reflected in the δ15N of the DIN pool in the ocean, N2 fixation (ε ~−1 ‰), nitrate uptake (ε ~+5 ‰), ammonium uptake (ε ~+5 ‰), water-column denitrification (ε ~+20 ‰), sedimentary denitrification (ε ~0 ‰), and anammox (ε ~+23.5 ‰) (Altabet and Francois, 1994; Brunner et al., 2013; Canfield et al., 2010; Galbraith and Sigman, 2008; Hoch et al., 1994, 1992; Lam et al., 2009; Waser et al., 1998). Of critical importance is the degree to which sedimentary organic matter reflects the δ15N of DIN. In modern settings, the δ15N of organic phases in sediments mirrors the isotopic composition of sub-euphotic zone nitrate (Thunell et al., 2004). Globally expansive meta-analyses of core top bulk sediment δ15N reveals patterns consistent with the known distribution of nitrogen cycle processes suggesting that δ15Nbulk is a reliable tracer for the δ15N of DIN (Robinson et al., 2012; Tesdal et al., 2013).

It is noteworthy that the δ15N of marine organic matter may be subject to alteration during diagenetic processes or influenced by allochthonous sources of nitrogen. Organic matter preserved in the Walnut Creek section has been shown to be predominantly derived from marine biomass (Murphy, Sageman, and Hollander, 2000; Tuite and Macko, 2013; Haddad et al., 2016), ruling out allochthonous terrestrial organic nitrogen contamination. Organic nitrogen can be subject to degradation under oxic to mildly reducing conditions within the water column and at
the sediment-water interface, the degree of enrichment depending primarily on water depth and residence time at or near the sediment-water interface (proportional to sedimentation rate).

Enrichment of $\delta^{15}N_{\text{bulk}}$ signals during particle sinking is more apparent in open ocean settings (Robinson et al., 2012; Tesdal et al., 2013), where $\delta^{15}N_{\text{bulk}}$ increases by $\sim+0.75\%$ km. However, alteration of $\delta^{15}N_{\text{bulk}}$ is minimal in shallow marine settings, and Late Devonian intracratonic basins of North America are inferred to have not been significantly deeper than 200 m (Algeo and Maynard, 1997; Jaminski et al., 1997; Potter et al., 1982). Applying the enrichment factor of $+0.75\%$ km to an assumed water depth of 200 m to our samples suggests enrichment in oxic settings of no more than $\sim0.15\%$, well below the average difference seen between grey and black shales in the IB ($+2.3\%$) and AB ($+1.6\%$ for KWEs and $+0.8\%$ for other black shales), though applying this factor, which was derived from open-ocean settings, to an epeiric sea system may imbue some minor uncertainty to the above comparison.

Shallow diagenesis can lead to a number of competing effects on the $^{15}N$ composition of sediments. Organic matter decomposition can result in deamination, or, the release of $^{15}N$-depleted ammonium from organic matter (Macko et al., 1993, 1987), which leads to an overall $^{15}N$-enrichment in sediments (Altabet, 1988; Lourey et al., 2003). However, the $^{15}N$-depleted ammonium is generally captured by clay minerals, leading to little net change in the overall $^{15}N$ composition of the sediments (Freudenthal et al., 2001). Degradation of labile amino acids is enhanced under suboxic conditions due to the preferential utilization of proteinaceous material by denitrifying bacteria (Van Mooy et al., 2002). Amino acids have been shown to be enriched by $\sim3\%$ on average compared to the bulk biomass of phytoplankton (Macko et al., 1987), and therefore their selective removal could potentially lead to a negative shift in sedimentary $\delta^{15}N_{\text{bulk}}$ values (Gaye-Haake et al., 2005; Prahl et al., 1997). However, this isotope effect is unlikely to
exceed the opposite effect associated with peptide bond breakage (~2.5 – 4 ‰) (Silfer et al., 1992) unless the amino acid fraction is removed in its entirety (Lehmann et al., 2002). Furthermore, even if the protein content of sedimentary organic matter was assumed to be relatively high, the complete removal of amino acid nitrogen would likely only result in up to a ~1 ‰ shift (Junium and Arthur, 2007), and would require a complete lack of degradation (and subsequent $\delta^{15}N_{\text{bulk}}$ enrichment) of other organic matter phases, which is an unlikely scenario. Therefore, it can be assumed that the effects of shallow diagenesis on the preserved $\delta^{15}N_{\text{bulk}}$ values presented here are minimal. Deeper diagenesis associated with metamorphism typically only results in minor alterations to $\delta^{15}N_{\text{bulk}}$ signals (Algeo et al., 2014; Imbus et al., 1992; Jia and Kerrich, 2004), and the rocks of the AB experienced only moderate thermal maturation (Haddad et al., 2016; Lash, 2017) and those of the IB, are thermally immature (conodont CAI of 1) (Day and Witzke, 2017), and are thus likely excluded from any deeper modification. Because the source of OM can be constrained, and thermal maturity is not a concern in the studied AB and IB sections $\delta^{15}N_{\text{bulk}}$ values can largely be considered to reflect primary nitrogen cycle processes (Altabet and Francois, 1994; Higgins et al., 2010; Robinson et al., 2012).

One final consideration that needs to be made in the interpretation of $\delta^{15}N_{\text{bulk}}$ records is the presence of inorganic N in organic matter lean samples (Calvert, 2004). The concern here being that as the overall percentage of marine organic matter decreases, the relative contribution of the inorganic N on $\delta^{15}N_{\text{bulk}}$ can be expected to increase, especially in clay rich sediments under oxygenated conditions. The inorganic N component comprises ammonium adsorbed to or fixed within the interlayers of clay minerals with the possibility that some of this ammonium was terrestrially derived (Calvert, 2004; Stevenson and Dhariwal, 1959). Organic matter degradation would subsequently increase the fraction of inorganic N in the sediments, potentially shifting
\( \delta^{15}N_{\text{bulk}} \). However, again the adsorption of the isotopically light ammonium derived from organic matter to the clays generally counteracts this effect (Freudenthal et al., 2001), leaving the sediments with little net change in \( \delta^{15}N_{\text{bulk}} \) values. If inorganic N did have significant impact on the \( \delta^{15}N_{\text{bulk}} \) of low TOC sediments, it can be expected that the \( \delta^{15}N_{\text{bulk}} \) of those sediments would display a strong mixing relationship between inorganic and organic N with changing TOC. Grey, organic lean shales from the Walnut Creek section show no statistically significant covariance between \( \delta^{15}N_{\text{bulk}} \) and %C (r = +0.20, p > 0.05, n = 25) or %N (r = −0.17, p > 0.05, n = 25) (Fig. 5). Grey shales from the TSC section do show a weak correlation between \( \delta^{15}N_{\text{bulk}} \) and %C (r = −0.42, p > 0.05, n = 22) and %N (r = −0.48, p < 0.05, n = 22), however the correlations are driven mostly by one or two anchor points on the edges of the data set. Removal of these outlying points removes the weak correlations observed (%C: r = −0.26, p > 0.05, n = 20; %N: r = −0.28, p > 0.05, n = 20) (Fig. 5) and therefore the influence of terrestrially derived inorganic N is likely not a significant concern for either of these study sites. The IGS-Sullivan site did not have enough grey shale data to analyze in this fashion (n = 2). Additionally, numerous studies have detailed the robust relationship between sedimentary \( \delta^{15}N_{\text{bulk}} \) values and water-column nitrogen cycling, even in organic lean oxic sediments (Freudenthal et al., 2001; Lehmann et al., 2002; Robinson et al., 2012).
Figure 4-5: Cross-plots of the $\delta^{15}\text{N}_{\text{bulk}}$ values of the organic lean grey shale from the Walnut Creek and TSC sites (IGS-Sullivan site had insufficient grey shale data to be statistically significant) with $\%\text{C}(\text{org})$ and $\%\text{N}$. Grey circles indicate outliers, and the upper r values for the TSC section are calculated with them included. The lower r values are calculated without the outliers (black circles only).
4.5.2.2 Nitrogen cycling in the Devonian AB and IB

The average $\delta^{15}N_{\text{bulk}}$ of the studied rocks of the AB and IB is significantly depleted in $^{15}$N compared to modern marine shelf and slope sediments (Tesdal et al., 2013) suggesting that the balance of nitrogen-cycle processes was fundamentally different. The IB and AB were largely isolated from the broader Late Devonian ocean by the expansive epeiric sea that covered much of Laurentia and were well-separated from nutrient sources that may have been supplied from the deep ocean, other than a narrow connection of the IB to the Rheic ocean to the south-west. Given the high ratio of seafloor area to water volume in the relatively shallow epeiric sea, sedimentary nitrate reduction may have been the primary sink for DIN during the more oxic conditions present during the deposition of grey shale surrounding the deposition of black/brown shales (Christensen et al., 1987; Galbraith and Sigman, 2008). Assuming a high fraction of benthic denitrification, nutrient limitation in the epeiric seaway likely favored relatively oligotrophic conditions. From an isotopic perspective, the direct effect of sedimentary denitrification on the $\delta^{15}$N of nitrate is negligible. However, replenishment of DIN deficits by diazotrophy would have pinned the $\delta^{15}$N of DIN near 0.0 ‰, as long as water-column denitrification remained limited (Algeo et al., 2014; Quan and Falkowski, 2009). The $\delta^{15}N_{\text{bulk}}$ values of grey shales in the IB (+2.5 to +4.0 ‰) and AB (+1.0 to +2.0 ‰) are consistent with deposition under generally oxic (Boyer et al., 2014) and oligotrophic conditions.

Circulation models of the Devonian Basin as well as the Mediterranean and Baltic seas provide context for interpreting the $\delta^{15}N_{\text{bulk}}$ differences between our respective sites and in the broader Devonian Basin. Algeo et al. (2007) proposed a ‘super-estuarine’ mode of overturning for the Devonian Basin in which deep waters entered the epeiric sea from the west and were entrained with less-saline surface waters derived from riverine sources to the east. The estuarine
circulation pattern resulted in net flow of surface waters from the eastern boundary of the seaway proximal to the AB westward toward the IB. In the context of this model, the difference of 2 - 3 ‰ in \( \delta^{15}N_{\text{bulk}} \) between the IB and AB (Fig. 2) may have been produced by a number of processes: differing relative influences of N\(_2\)-fixation and nitrate reduction in each basin; the advection and progressive uptake of \( ^{15}N \)-depleted DIN as surface waters advanced from east to west (Fig. 1) similar to Rayleigh like distillation; or increased sources of terrestrially derived DIN or phosphorus in the AB from Appalachian highlands that stimulated diazotrophy not seen in the IB. Alternatively, it is possible that productivity in the IB was fueled by DIN sources derived primarily from the Rheic Ocean to the southwest whereas the more isolated, silled AB functioned as described above. Further careful study of the sources of deep water by Nd or other similar water mass tracer methods (e.g. Scher et al., 2015) will elucidate the role of circulation in establishing \( \delta^{15}N \) values. Indeed, the \( \delta^{15}N_{\text{bulk}} \) difference between AB and IB was likely the net effect of all of these processes.

Paleogeographic considerations of Laurentia (Fig. 1) suggest that rivers were important sources of nutrients to the IB and AB. The expansion and stabilization of the terrestrial biosphere would have provided an enhanced flux of DIN and P to the global ocean (cf. Algeo and Scheckler, 1998) and may have been particularly important in epeiric seas like the AB. Though modern rivers systems are certainly imperfect analogs for those draining the early Devonian terrestrial biosphere, they can provide some perspective on their impacts to marine systems. Nutrients delivered to the western Atlantic Ocean from the Amazon River substantially increase productivity within its mesohaline plume. As DIN supplies diminish, productivity is maintained by increasing diazotrophy in the North Atlantic more than 1000 km from the point of outflow (Subramaniam et al., 2008). The impact of riverine nutrients would have been most significant in
the AB. Moreover, low $\delta^{15}$N$_{\text{bulk}}$ and higher %C values of the studied AB samples reflect the net effect of higher nutrient supply, and possibly, increased diazotrophy.

The described observation that black/brown shales of both basins are $^{15}$N-depleted and statistically distinct from assimilated grey shales (Fig. 3, and supplement Table S2) suggests a robust facies dependence. Similar apparent facies control has been described from the sapropels of the Mediterranean (Higgins et al., 2010; Milder et al., 1999) and Baltic seas (Bianchi et al., 2000). In these more recent examples, $^{15}$N-depletion to near 0 ‰ accompanied sapropel deposition and occurred under predominantly anoxic water column conditions (Fig. 3). The transition into and out of sapropel deposition in the Eastern Mediterranean and Baltic seas and oscillations of $\delta^{15}$N$_{\text{bulk}}$ were controlled by hydrographic changes in the respective basins (Bianchi et al., 2000; Higgins et al., 2010). Freshwater inflow to the Baltic and Eastern Mediterranean basins established estuarine circulation and increased phosphorus-flux that stimulated productivity, fostered anoxia, and allowed sapropel deposition and preservation. The estuarine model of circulation and biogeochemical feedbacks associated with sapropel deposition are similar to conditions proposed for the Mid-Cretaceous North Atlantic (Trabucho Alexandre et al., 2010) and the Devonian epeiric sea (Algeo et al., 2007; Murphy et al., 2000).

The biogeochemical mechanism of $^{15}$N-depletion in the AB and IB during black/brown shale deposition, if similar to the Eastern Mediterranean and Baltic seas (Bianchi et al., 2001; Higgins et al., 2010), would have been largely dependent on increased phosphorus supply and regeneration that stimulated diazotrophy. However, our model for $^{13}$C-depletion during black shale deposition suggests that organic matter production below the chemocline, or utilization of DIN derived from the chemocline may have been important factors in the nitrogen cycle as well. Like organic carbon, the nitrogen in surface sediments from the anoxic Framvaren Fjord have
δ\textsuperscript{15}N\textsubscript{bulk} values that are ~2 \textperthousand lower than particulate organic matter (POM) from surface waters (Velinsky and Fogel, 1999). The δ\textsuperscript{15}N of POM decreases significantly within and below the chemocline suggesting that addition of new organic matter from depth was an important fraction of sedimentary organic matter. Similar trends are also observed in other anoxic basins with relatively shallow stratification such as Green Lake, in Fayetteville, New York (Havig et al., 2017; Fulton, 2010) and provide a reasonable model linking isotopic depletion of nitrogen and carbon in the AB and IB during the KWEs.

4.5.3 Persistent, versus punctuated anoxia

The geologic and geochemical evidence suggests that the IB and AB experienced oscillating redox conditions over the study interval. Some discrete black shale beds that contain a benthic macrofauna and bioturbation indicative of at least intermittent oxic conditions, also contain pigment biomarker evidence for photic zone euxinia (Boyer et al., 2014; Brown and Kenig, 2004; Haddad et al., 2018, 2016; Schieber, 2009), supporting shorter term redox oscillations during black shale deposition. Redox variability is further supported at Walnut Creek by pyrite framboid size distributions that indicate euxinia in either the water column or at the sedimentary redox interface (Lash, 2017). The episodic or seasonal relaxation of stratification through variations in winds or basin hydrography would have allowed for the mixing of deeper waters in these relatively shallow basins.

The impact of redox oscillations on the nitrogen and carbon stable isotopic record can be significant. Mixing of isotopically depleted DIC depressed δ\textsuperscript{13}C\textsubscript{org}, and ammonium of sufficiently high concentrations may have allowed for incomplete utilization by primary producers and lower δ\textsuperscript{15}N\textsubscript{bulk} during KWEs. Ammonium is present in high concentrations in the
anoxic regions of stratified water columns (Fulton et al., 2012; Havig et al., 2017; Velinsky and Fogel, 1999; Voß et al., 1997), and would have been available for use at/below the chemocline or within the photic zone during mixing events (cf. Higgins et al., 2012). Ammonium is preferentially used as a nitrogen source for phytoplankton growth over nitrate, as it is already in a biologically useful oxidation state and does not require energy intensive reduction as is the case with nitrate (Eppley et al., 1969). Biomass produced utilizing ammonium would therefore have had a significant contribution to the particulate nitrogen pool even at lower relative concentrations (Harrison et al., 1996; Pennock et al., 1996; Probyn et al., 1996). Also, ambient ammonium inhibits the expression of the genes that regulate the proteins used in assimilatory nitrate reduction and transfer of nitrate across cell membranes of cyanobacteria (Flores and Herrero, 2005) thereby enhancing the influence of ammonium on microbial growth. The greater prevalence and utilization of ammonium could have driven $^{15}$N-depletion observed in black/brown shales as ammonium has a much larger assimilatory fractionation than does nitrate (Hoch et al., 1994, 1992; Pennock et al., 1996; Waser et al., 1998), and has the potential to result in more isotopically depleted biomass being delivered to the sea floor, assuming incomplete utilization.

It should be noted that this model is subtly different from that proposed for Mesozoic OAEs. The lower $\delta^{15}$N$_{\text{bulk}}$ (<0 ‰) typical of OAE deposits is attributed to incomplete ammonium utilization by phototrophs in the euphotic zone. The $\delta^{13}$C$_{\text{org}}$ records from OAEs typically follow the exogenic carbon cycle and are largely independent of $\delta^{15}$N (Higgins et al., 2012; Junium and Arthur, 2007). This relationship suggests that neither the isotopic fingerprint of the chemocline nor production from within it were of a sufficiently large fraction of the preserved organic matter during OAEs to depress $\delta^{13}$C$_{\text{org}}$ despite persistently anoxic conditions. Within the AB and IB
across the Frasnian-Famennian boundary however, the coupled depletion in $\delta^{15}$N$_{\text{bulk}}$ and $\delta^{13}$C$_{\text{org}}$ indicates that a significant portion of organic matter was being produced either within/below the chemocline or utilizing chemocline-derived nutrients during upwelling events. The proportion of chemocline-derived organic matter would not need to be overly large to induce the depletions observed at the studied locations. Indeed, Haddad et al., (2016) and de la Rue et al., (2007) show a predominance of eukaryotic biomass preserved at Walnut Creek and at an IB site in Indiana. However, both basins display broad evidence for chemocline productivity (Brown and Kenig, 2004; Haddad et al., 2018; Haddad et al., 2016), indicating that there was some discernable measure of shallow chemocline-derived organic matter incorporated into these sediments. Due to the nature of the fractionation factors involved with ammonium uptake, as well as the potential presence of isotopically depleted DIC residing at/below the chemocline (as well as the potentially larger carbon isotope fractionation factor associated with certain chemocline dwelling organisms), the percentage of chemocline derived POM required to effect a $\sim$1 - 2 % shift in carbon isotopes, and a 1 - 3 % shift in nitrogen isotopes could be relatively small. Assuming that average photic zone biomass has a $\delta^{13}$C$_{\text{org}}$ of $\sim$26 % (peak excursion value in grey shale of Walnut Creek, Fig. 2) and that chemocline organic matter is as low as $-$40 % (cf. Havig et al., 2017), a 1 - 2 % offset could be achieved with only 7 - 15 % of the organic matter being derived from the chemocline. Lastly, the proportion of organic matter preserved in sediments derived from the chemocline versus produced during upwelling events, would be dependent on the depth of the chemocline relative to the euphotic zone, as well as the frequency of upwelling/mixing events. Both of these factors would have been contingent upon the climatic conditions associated with the KWEs and the Late Devonian as a whole.
4.5.4 Insights on the Kellwasser Events

The KWEs were global scale perturbations to the carbon cycle and marine environments (Joachimski et al., 2002; Racki, 2005; Racki et al., 2018). The black shales associated with the KWEs in the AB and IB accumulated over a relatively short interval of time during a quiescent phase of the Acadian orogeny (Lineback, 1970; 1980; Sageman et al., 2003) and are associated with rapid changes in sea level (Bond and Wignall, 2008; Day and Witzke, 2017). Despite the relatively narrow spatial and temporal scope of our study, our data can provide perspective from which we can assess the proposed geochemical and paleoclimatic mechanisms proposed for the Frasnian-Famennian biotic crisis and the controls on black shale deposition during this time. Here we focus on the role of climatic and sea level change, and potential effects on circulation and nutrient cycling within the AB and IB.

The time period encompassing deposition of the KWEs was significantly warmer than the previous Eifelian to mid-Frasnian period (Joachimski and Buggisch, 2002; Joachimski et al., 2004). Water temperatures calculated from conodont apatite and brachiopod and bivalve calcite $\delta^{18}O$ from sites of similar paleo-latitude to the IB and AB (between ~25 and 30 °S), reveal a temperature range of ~26 - 32 °C (Chen et al., 2002; Izokh et al., 2009; Joachimski and Buggisch, 2002; Joachimski et al., 2004; van Geldern et al., 2006; Zheng et al., 1993). Superimposed over this interval of warmth, marine temperatures increased, coincident with the initial increase in $\delta^{13}C$ that signals the initiation of the KWEs globally, and the decrease in $\delta^{15}N_{bulk}$ observed in the AB and IB, followed by a 5 - 7 °C cooling trend (Joachimski and Buggisch, 2002) (Fig. 4). Model results from Goddéris and Joachimski (2004), suggest that the ~3.0 ‰ positive $\delta^{13}C$ excursions during the KWEs would have required productivity induced
drawdown of atmospheric CO\textsubscript{2} from \textasciitilde3000 to 1560 ppm. The organic carbon burial mediated decrease in CO\textsubscript{2} is consistent with δ\textsuperscript{18}O evidence for cooling.

The increased productivity necessary to halve CO\textsubscript{2} required as much as a 40 \% increase in phosphorus delivery to the ocean (Goddéris and Joachimski, 2004). From the context of the AB and IB, initial warming would have enhanced continental weathering, and increased freshwater delivery to the basin (De Vleeschouwer et al., 2014), which would have been accompanied by weathering derived phosphorus and terrestrially sourced DIN. The strengthening of halostratification and intensification of the ‘superesturarine’ circulation, both considered fundamental to persistent oxygen deprivation (Algeo et al., 2007), could have forced the expansion of benthic anoxia and black shale deposition. As detailed above, chemocline dynamics in the IB and AB altered the average water column position of organic matter production, inducing a reduction of δ\textsuperscript{15}N\textsubscript{bulk} and δ\textsuperscript{13}C\textsubscript{org} (Figs. 2 and 4). Ultimately, a cooling climate was sufficient to enhance basinal oxygenation and curtail the strength of thermohaline density stratification, leading to the termination of black shale deposition associated with the KWEs (cf. Arthur et al., 1988; Freeman and Hayes, 1992; Sinninghe Damsté et al., 2010) and a return to a surface production and nitrate dominated nitrogen cycle (Fig. 4). However, this did not occur until temperatures were significantly lower than prior to the initiation of the KWEs (Fig. 4) and suggests that black shale deposition was not solely dependent upon temperature.

The continuation of black shale deposition in the face of cooling may be linked to the internal phosphorus-cycling dynamics of the studied sections where phosphorus is regenerated from sedimentary organic phases and metal oxides (Ingall et al., 1993; Van Cappellen and Ingall, 1994). High C\textsubscript{org}/P ratios in the organic matter-rich shales of the IB (Ingall et al., 1993) and AB (Sageman et al., 2003; Tuite and Macko, 2013) are consistent with efficient phosphorus
recycling that was probably an important factor in the enhancement of primary production. This model is similar to the phosphorus-cycle models described for Mesozoic OAEs (cf. Mort et al., 2007; Adams et al., 2010), which appears to reflect phosphorus regeneration fostered ‘productivity-anoxia feedbacks’ (Van Cappellen and Ingall, 1994). Despite evidence for episodes of punctuated oxygenation in the AB and IB sections studied here (Boyer et al., 2014; Brown and Kenig, 2004; Haddad et al., 2018, 2016; Lash, 2017; Schieber, 2009), it is clear that episodic ventilation was insufficient to depress $C_{\text{org}}/P$ and did not limit phosphorus-regeneration (Sageman et al., 2003; Tuite and Macko, 2013). On the contrary, episodic overturn would have returned sub-chemocline phosphorus and DIN to the surface, thereby stimulating primary productivity and black shale deposition at the studied sections despite decreasing temperatures.

The presence or absence of a ‘productivity-anoxia feedback’ (Van Cappellen and Ingall, 1994) could also help explain different rates of organic enrichment in various basins around the world. Productivity at sites with modest organic enrichment (such as those in Australia and China; (Fig. 1)) would have been augmented by the greater influx of terrestrial phosphorus derived from initial warming but may not have had the requisite basinal geometry/hydrographic features to develop water-column anoxia and initiate phosphorus regeneration, potentially like the AB and IB sections studied here. This distinction can even be made between the AB and IB during the LKE, as the IB sites examined here did not develop high TOC brown shales during the LKE interval, highlighting a disparity in the extent of expansion of anoxic conditions during the two events. This difference could be related to a differing degree of initial warming during the KWEs, as the LKE does have slightly lower inferred sea surface temperatures than the UKE in conodont apatite (Joachimski and Buggisch, 2002), or to differential effects of sea level rise.
The episodic expansion of low-oxygen waters into shallow shelf systems in tandem with cooling would have not only placed extreme stress on existing shallow, warm water fauna (e.g. Murphy, Sageman, and Hollander, 2000; Copper, 2002), but also presented an environment that was inhospitable to new colonization within the AB and IB. Such an oscillating redox regime has also been proposed for sites in the Holy Cross mountains of Poland (Kazmierczak et al., 2012), and if other sites at tropical latitudes experienced similar conditions, this could have contributed the global signal of depressed origination rates (Bambach, 2006). Paleolatitude is of particular significance because the tropics are thought to act as the locus of origination from which new taxa spread (Jablonski et al., 2006), meaning that mass depletion (Bambach et al., 2004) occurring in the tropics could propagate globally. Applying coupled high resolution $\delta^{15}$N and $\delta^{13}$C$_{org}$ isotope systematic studies, such as those presented here, along with other paleo-redox proxies (Boyer et al., 2014; Brown and Kenig, 2004; Haddad et al., 2018, 2016; Lash, 2017; Schieber, 2009), with a particular focus on other tropical-subtropical platform localities could help further elucidate the distribution and effect of oscillating redox conditions on the Frasnian-Famennian biotic crisis.

4.6 Conclusions:

The data presented here provide evidence for the influence of chemocline-derived nutrients/production on the $\delta^{15}$N$_{bulk}$ and $\delta^{13}$C$_{org}$ of preserved organic matter in the AB and IB during the Frasnian-Famennian biotic crisis. The observed low $\delta^{15}$N$_{bulk}$, coupled with $\delta^{13}$C$_{org}$ depletions within black shales are consistent with elevated utilization of isotopically depleted nutrients, incomplete utilization of highly concentrated nutrients either at/below a shallow chemocline/in surface waters during episodic overturn events, or some combination of these two
scenarios. Brief warming at the onsets of the LKE and UKE, and subsequent increased freshwater and phosphorus delivery to the intracratonic basin system may have led to conditions that fostered nutrient trapping, elevated primary productivity, and a shoaling of the chemocline. Enhanced productivity in the AB, IB, and globally led to CO₂ drawdown and climatic cooling, and eventual termination of brown/black shale deposition associated with the KWEs. The episodic expansion of low-oxygen waters into shallow shelf systems, coupled with a cooling climate would have placed extreme stress on organisms adapted to warm, oxic conditions, and may have presented environments inhospitable to new colonization.

4.7 Acknowledgments:

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APPENDIX A1: CHAPTER 2 SUPPLEMENTARY MATERIALS

Figure A1-1: Molecular structures referenced in Table 1 and Fig. 2.

A: 

B: 

C: 

D: 

E: $R_1 = \text{CHO} \quad R_2 = \text{CH}_3$

F: $R_1 = \text{C}_2\text{H}_3 \quad R_2 = \text{CHO}$

G: $R_1 = \text{C}_2\text{H}_3 \quad R_2 = \text{CH}_3$

H: $R_1 = \text{OH} \quad R_2 = \text{CHO}$

I: $R_1 = \text{H} \quad R_2 = \text{CHO}$

J: $R_1 = \text{H} \quad R_2 = \text{H}$

K: 


**Figure A1-2:** Linear correlation between derived UV A irradiance as % of surface irradiance, and scytonemin concentration at each sample site.

\[
y = 0.053315 + 0.038747x \quad R^2 = 0.9159
\]
APPENDIX A2: CHAPTER 3 SUPPLEMENTARY MATERIALS

Figure A2-1: Crossplots of geochemical data, and C/N ratios with depth

A: Crossplot of δ¹³C (%) vs. TOC (%) with a correlation coefficient of r = 0.03, p = >>0.05

B: Crossplot of δ¹³C (%) vs. C/N with a correlation coefficient of r = 0.39, p = < 0.01

C: Crossplot of δ¹⁵N (%) vs. %C with a correlation coefficient of r = 0.05, p = >>0.05

D: Crossplot of δ¹⁵N (%) vs. %N with a correlation coefficient of r = 0.22, p = > 0.05

E: Crossplot of δ¹⁵N (%) vs. C/N with a correlation coefficient of r = 0.35, p = < 0.05

F: Core depth vs. C/N plot
## APPENDIX A3: CHAPTER 4 SUPPLEMENTARY MATERIALS

### Table A3-1: Data used in this study

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<th>$\delta^{15}$N ($%$)</th>
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Table A3-1: Black shales are shaded; grey shales are not. Kellwasser events are in bold, with the Lower Kellwasser Event always occurring first in the table. Data from the Pipe Creek shale at
Walnut Creek with * next to the stratigraphic height are derived from Lash (2017). We took the upper most value reported by Lash (2017) for the Pipe Creek (~27 ‰), and linearly interpolated to it from the uppermost sample of our data, at the average sampling density of our data in the Pipe Creek (~2 cm). This was done in order to give proper weight to those data when determining the average δ^{13}C_{org} for the Pipe Creek and comparing it to the bounding grey shales.

Figure A3-1: Q–Q plots of δ^{15}N data from each lithology studied from the AB and IB.

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<td>IB GS 0.14762</td>
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Figure A3-1: Open circles represent data from table 1. Plots where most open circles lie on, or close to solid line are considered normal. Table inset in bottom right shows Shapiro Wilk p value statistics for each distribution. Values larger than 0.05 indicate a failure to reject the null hypothesis of normality, indicating that the data is normally distributed.
Table A3-2: Kolomorgorov–Smirnov test statistics

Appalachian Basin (AB)

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<th>KW – GS</th>
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Illinois Basin (IB)

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Mediterranean Sea (Milder et al., 1999. Site 969)

<table>
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<td>D_{max}</td>
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Table A3-2: D_{crit} indicates threshold value that must be exceeded in order to say two distributions are statistically distinct. D_{max} is the maximum difference between the cumulative distribution function of the compared distributions. If D_{max} > D_{crit}, the distributions can be said to be distinct. A D_{max} of 1.000 indicates no overlap between distributions.
Figure A3-2: Macerates from Walnut Creek section

Figure A3-2: Top two pictures are taken from the Angola grey shale, ~53 cm below the Pipe Creek/Lower Kellwasser Event black shale. Bottom left is taken from the Pipe Creek/Lower Kellwasser interval, 13 cm above Angola–Pipe Creek contact, and the bottom right from 21 cm.
Benjamin Thornton Iglar Uveges
Curriculum Vitae

EDUCATION:

2018  PhD. Earth Sciences, Syracuse University: December 2018
Title: Characterizing ancient chemoclines through the use of pigment biomarkers and sedimentary stable isotope signatures

2013  B.Sc. Chemistry, McGill University

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Present: Visiting Assistant Teaching Professor. Syracuse University Department of Earth Sciences
2018: Part time instructor: Syracuse University Department of Earth Sciences
2015-2018: Teaching assistant: Syracuse University Department of Earth Sciences
2015-2018: Research assistant: Syracuse University Department of Earth Sciences

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2016  UNOLS Chief Scientist Training Cruise ($1500)
2015  Syracuse University Earth Science Department Chairs Award
2013-2015  Syracuse University Graduate Fellowship

PUBLICATIONS:

https://doi.org/10.1016/j.palaeo.2018.05.031

https://doi.org/10.1038/s41467-018-05486-w

Uveges, B.T., Junium, C.K., Teece, M.A., Fulton, J.M. Environmental controls on pigment distributions in the freshwater microbialites of Fayetteville Green Lake. Organic Geochemistry (Accepted)