Pigment and Geochemical Markers of Past and Present Cyanobacterial Presence in the Southwestern Lake Superior Region

by Lily Karg

In the summer of 2012, the oligotrophic waters of Lake Superior experienced the region's first cyanobacterial bloom along the west shoreline of the Wisconsin Apostle Islands. While cyanobacterial blooms are common in other Laurentian Great Lakes and warmer inland lakes, this was the first event recorded in Lake Superior. In contrast, Siskiwit Lake, WI, which drains into Lake Superior near the bloom location, has reported semi-annual cyanobacterial blooms each summer. This study focused on a more recent 2021 bloom in Siskiwit Lake, along with the sediment deposits in Siskiwit Lake and Siskiwit Bay, near the first 2012 bloom event. Using elemental and isotopic indicators (%OC, %N, and $\delta^{13}C$), and High-Performance Liquid Chromatography (HPLC), the contribution of organic carbon sources over time in these locations was analyzed and compared with the carotenoid and pigment portrait of the 2021 bloom in Siskiwit Lake. Findings suggested that OC sources in the sedimentary record of Siskiwit Bay were primarily lacustrine algae, demonstrating periods of high algal matter productivity, while Siskiwit Lake exhibited higher terrestrial organic matter input in comparison. Furthermore, carotenoid pigments identified in the Siskiwit Lake bloom indicated a large presence of cyanobacteria amongst other algae, corroborating the presence of cyanobacteria paleo-indicators within the sediment. With this data, future investigations of algal pigments in the Lake Superior lacustrine deposits will provide additional reconstruction of high cyanobacteria productivity intervals and identify potential bloom periods in the region's history.

I. Introduction

Cyanobacteria, also known as blue-green algae, are ancient photosynthetic bacteria that use sunlight and carbon dioxide (CO_2) to produce biomass (Paerl, 2017). These organisms are responsible for stimulating the oxidation of Earth's atmosphere billions of years ago and are naturally present in almost all aquatic ecosystems (Huisman et al., 2018). Some cyanobacteria species, such as those within the *Dolichospermum* genus, have also developed the unique ability to fix atmospheric nitrogen (N_2) over their extensive evolutionary history (Huisman et al., 2018). Nitrogen fixation allows cyanobacteria to capture atmospheric N_2 gas and convert it into ammonia through catalysis via the nitrogenase enzyme complex, giving them competitive advantage over other non-nitrogen-fixing phytoplankton communities (Huisman et al., 2018).

As cyanobacteria have adapted to survive in adverse conditions, a warm and chemically nutrient-rich environment promotes their prolific biomass production (Huisman et al, 2018). When favorable conditions stimulate cyanobacteria to rapidly reproduce, they form thick mats on the surface of the water that are dense enough to extinguish the photosynthetic activities of aquatic plants (Huisman et al, 2018). The ultimate degradation of this large amount of biomass also deprives fish and benthic invertebrates of oxygen within the water column (Huisman et al, 2018). Furthermore, some cyanobacteria blooms can produce harmful toxins that are not only dangerous to the aquatic ecosystem but also humans, livestock, and other organisms that encounter them (Huisman et al., 2018; Sterner et al., 2020).

Recent accumulation of cyanobacteria blooms in Lake Superior, the largest and most oligotrophic of the Laurentian Great Lakes, have raised concerns for the health of the region's waters, as well as questions about the connection between these bloom events and climate change. In the summer of 2012, Lake Superior experienced the first recorded cyanobacterial bloom along the western shoreline of the Wisconsin Apostle Islands. While cyanobacterial blooms are common in other Laurentian Great Lakes and warmer inland lakes, this was the first event documented in Lake Superior. Several other blooms have occurred periodically in the lake since 2012, and such uncharacteristic algal proliferation has prompted investigation into the paleolimnology record within the lake's sedimentary deposits.

Sediment geochemistry and phytoplankton photopigments are both proxies used in the interpretation and reconstruction of past ecological conditions of lake environments. More specifically, organic matter (OM) and carotenoid biomarkers preserved within the sedimentary record can be analyzed for indicators of different algal communities. Through chemical analysis, elemental carbon to nitrogen ratios (C:N) in correlation with isotopic carbon signatures ($\delta^{13}C$) can be used to distinguish between primary sources of OM: C4 land plants, C3 land plants, marine algae, and lacustrine algae (Meyers, 1994). These signatures can then be paired with biomarker identification and quantification to reconstruct the organismal composition of historic ecosystems.

According to a review by Meyers (1994), algae lack the abundant amounts of cellulose that vascular land plants possess. These compositional differences are reflected in their carbon to nitrogen ratios: C:N ratios between 4 and 10 are typically seen in algae, while ratios greater than or equal to 20 are observed in vascular land plants (Meyers, 1994). Diagenesis related degradation of OM has been shown to alter C:N ratios in soils but remain reliably preserved in underwater environments (Meyer, 1994). Furthermore, carbon isotope signatures ($\delta^{13}C$) are used as markers of land plant types and differentiating marine and continental plant sources (Meyers, 1994). $\delta^{13}C$ signatures that fall within the -25‰ to -30‰ range are typically identified as freshwater OM, identified by Meyers in 1990 and 1992. It should be noted, however, that these signals are usually indistinguishable from C^3 land plants (Meyers, 1994). C4 land plants, on the other hand, show much more enriched $\delta^{13}C$ signatures, around -10‰ to -14‰ (Meyers, 1994).

Cyanobacteria synthesize carotenoids, which are hydrophobic isoprenoid compounds that act as both accessory light-harvesting pigments and protection against harmful photooxidation (Tóth et al., 2015). The specific carotenoid pigments synthesized by different types of cyanobacteria can be used to identify specific taxon present in both paleo and contemporary blooms. Generally, the literature reports that freshwater cyanobacteria genera display carotenoid suites predominantly composed of echinenone and canthaxanthin, as well as the photoprotective pigments myxoxanthophyll and zeaxanthin (Hertzberg et al., 1971; Hirschberg & Chamovitz, 1994). In conjunction with elemental and isotopic analysis, pigment suites can be surveyed to identify the organic material sources within historic and present environments.

A. Using Specific Photopigments as Biomarkers of Phytoplankton Communities

The analysis of organic material within natural samples uses several proxies as source indicators. In studies of sediment and water surface samples, photosynthetic pigment and carotenoid biomarkers can be separated and used to identify specific taxon present in both paleo and contemporary environments (Hertzberg et al., 1971; Hirschberg & Chamovitz, 1994). Their quantification also serves as a means to interpret productivity. Highly referenced research, such as Bianchi et al. (1991), indicates that concentrations of chlorophyll-*a* and total photopigments relative to total organic carbon within a sample site are specific measures of autochthonous community contributions.

Several instances have shown that this data can be used to infer bloom periods within the sediment record of lakes (Baustain et al., 2020; Hobbs et al., 2021; Leavitt, 1993). Cyanobacteria have typically been identified based on the presence of myxoxanthophyll (1 & 2), echinenone, canthaxanthin, zeaxanthin, and aphanizophyll (Descy et al., 2000 Hertzberg et al., 1971; Hirschberg & Chamovitz, 1994; Schlüter et al, 2018). Detection of these markers within the sedimentary record has suggested the presence of algal communities consisting of cyanobacteria in several studies, and further interpretation of their concentrations has provided insight into the context of blooms events as we experience them today (Baustain et al., 2020; Bianchi et al., 1991; Hobbs et al., 2021; Leavitt, 1993; O'Bierne et al., 2017).

Additionally, carotenoid concentrations in relation to total pigment composition and/or chlorophyll-*a* concentrations have been used to correspond characteristic ratios to algal genera (Jeffery et al., 2011; Schlüter et al., 2018; Hertzberg et al., 1971; Egeland, 2016). While some pigments are especially unique to individual microorganism classes, several are shared, including diadinoxanthin, zeaxanthin, and neoxanthin (Bianchi & Canuel, 2011; Egeland, 2016; Hirschberg & Chamovitz, 1994). Methods of distinguishing between the sources of these pigments, which are found in several organisms, continues to be researched and improved. The analysis of pigment marker to Chl-*a* ratios has thus been used to further differentiate between types of cyanobacteria or other phytoplankton groups and their respective contributions in both culture and field measurements through a variety of techniques, as well as assessing post-depositional pigment preservation (Descy et al., 2000; Hobbs et al., 2021)

B. The Reliability of Pigments as Biomarkers

Natural systems are often difficult to study due to their dynamic interactions and complex cycles, emphasizing the need for examination of the reliability of biomarkers in analytical assessments. Runoff, winds, current, and other physical forces influence the exchange of water in estuaries and coastal regions (Baustian et al., 2020; Reinl et al., 2020; Schlüter et al., 2014). Other environmental changes can alter the biochemical interactions within the water column, as well (Schlüter et al., 2014). These influences subject any organic biomarkers, including photopigments, to degradation forces. As they are susceptible to decay through *in situ* activities (including oxidation and herbivory) and post-depositional degradation, some studies have claimed that relative pigment abundance should not be relied on as an accurate reflection of phytoplankton community composition in the fossil record (Leavitt, 1993; Ruess et al., 2005). Further degradation can occur before, during, or post extraction from both sediments and water surface samples (Hirschberg et al., 1994; Hertzberg et al., 1971; Chen et al., 2001). Moreover, other biochemical processes *in vivo*, such as chlorophyllase activity, can create problems when analyzing chlorophyll (Jeffrey & Hallegraeff, 1987). Despite these selective losses, studies focusing on individual pigment fossil profiles with respect to site-specific historical patterns show that population interaction interpretations are acceptable (Leavitt, 1993; Baustian et al., 2020).

Recent work by Baustian et al. (2020) has particularly demonstrated the utilization of paleolimnological indicators of long-term ecological changes within the Laurentian Great Lakes Basin to guide management practices towards site-specific measures. Their quantification of chlorophyll-a, carotenoid, and pigment derivatives was combined with elemental and isotopic analysis, in the first regional appraisal of the impacts of invasive mussel species and eutrophication via the fossil record (Baustian et al., 2020). Parallels between their results and those of related studies, correlations with nutrient level monitoring, and quality assurance of their data, both geochemical and pigment biomarkers were found to be applicable indicators of human impact on water quality (Baustian et al., 2020).

Similarly, another recent study conducted by Hobbs et al. (2021) employed analogous methods in the investigation of the sedimentary record of a parkland lake known to host recurring harmful algal blooms. Hobbs et al. (2021) provided historical context of the algal community within the lake system and identified impacts of the surrounding parkland and farming. Both of these independent studies by Baustian et al. (2020) and Hobbs et al. (2021) substantiated that despite natural interferences, pigments served as useful molecular markers with long-term monitoring and considerate examination of sedimentary records in site-specific interpretations.

Another study by Descy et al. (2000) reported reliability in their methods of algal biomass prediction. Their data advocated that marker pigment to Chl-*a* ratios showed enough agreement with calculated values to corroborate acceptable estimations of phytoplankton concentrations (Descy et al., 2000). Apart from their own empirical analysis, a coupled review examined several other techniques of phytoplankton biomass quantification, with instances of unreliability reportedly due to light and nutrient availability changes by and cross-over within taxonomic classifications (Descy et al., 2000). Despite underlying limitations revealed through their review, Descy et al. (2000) detailed promising results from their own findings and other studies using CHEMTAX estimation of algal biomass and those utilizing the relationship of carbon biomass and xanthophyll concentrations.

C. Anthropogenic Climate Change in Connection with Cyanobacteria Productivity

Anthropogenic activities not only impact the use of pigment biomarkers in assessments of contemporary and historic phytoplankton communities, as reviewed in previous sections, but also the dominance of cyanobacteria in algal communities. Some studies have investigated potential drivers for increased cyanobacteria productivity in connection with climate change (Baustian et al., 2020; Hobbs et al., 2020; O'Bierne et al., 2017; Paerl & Paul, 2012; Reinl et al., 2020). As global temperatures warm and pollution from human activities contaminate water bodies and systems, for example, cyanobacteria and other phototrophic organisms easily proliferate (Paerl & Paul, 2012; Reinl et al., 2020). Moreover, changes in cyanobacterial dominance in aquatic communities as a result of climate conditions such as surface water temperatures, ice-free periods, nutrient loading, vertical stratification, and carbon dioxide availability have been correlated with anthropogenic influences (O'Bierne et al., 2017; Paerl & Paul, 2012).

The effects of climate change on the primary productivity within aquatic ecosystems have been observed through studies of bulk OM and pigment content within the sedimentary deposits of varying lakes (Leavitt, 1993; Reinl et al., 2020; O'Bierne et al., 2017; Paerl & Paul, 2012). An investigation by O'Bierne et al. (2017) reported that a rapid increase in autotrophic productivity within Lake Superior was found to be correlated to higher surface water temps and longer ice-free periods as a result from anthropogenic climate change, with the caveat that individual elemental markers should not be interpreted alone but in relation to other indicators. They proposed that changes in climate regimes observed recently within the lake system have been more influential than those experienced historically (O'Bierne et al., 2017). A similar review was published by Hobbs et al. in 2020, evaluating algal pigment and genetic biomarkers of a lake within the Olympic Peninsula that has been experiencing uncharacteristic cyanobacteria blooms. Their findings also suggested that climate change influences on nutrient inputs and cycling have altered the ecological development of toxic cyanobacteria species (Hobbs et al., 2020).

As mentioned previously, work by Baustian et al. (2020) had also investigated an array of damaging anthropogenic stressors on the Great Lakes region. Further synthesis of previously conducted studies highlighted damaging human interactions with freshwater ecosystems, including but not limited to the introduction of invasive species, agricultural development, suburbanization, and industrial chemical and biological pollutants (Baustian et al., 2020). Due to issues of eutrophication, algal proliferation, pollution, and suffering fish and wildlife populations, their study sites (within the western area of Lake St. Clair, Michigan, part of the Laurentian Great Lakes system) have been recognized by the Great Lakes Water Quality Agreement as an Area of Concern since 1987 (Baustian et al., 2020). Along with other studies within the nearby regions, the assessment of anthropogenic and climatic variables associated with declining water quality by Baustian et al. (2020) exemplify the exigency for further evaluation of the watershed's health and potential mitigation solutions.

D. Knowledge Gaps and Current Work

As has been reviewed here, a composite of internal and external factors influences both the incipient development of cyanobacterial communities and the use of biomarkers to identify them in aquatic ecosystems. Studies have evidenced that cross-examination of these interactions allows for analysis of contemporaneous and fossilized chemical and biological markers to reconstruct the ecological profiles of cyanobacteria, interpret their compositional changes over time, and infer the relationship of anthropogenic climate change in the accumulation of harmful bloom events. Gaps in the literature call for further research of bloom event triggers and prediction frameworks, as well as the taxonomic record of specific phytoplankton species, markedly in the Great Lakes. Environmental assessments have also been recommended to be performed and/or taken into consideration specific to study sites when interpreting data. Considering Lake Superior, investigation into the lacustrine deposits and current phytoplankton communities will issue the groundwork for a comprehensive exploration of the lake's dynamic biogeochemistry and how a changing climate could adversely affect it.

This study examined photopigment and geochemical markers of past and present cyanobacterial presence in the southwestern Lake Superior region in response to concerns around recent bloom events. Sediment cores were collected from Siskiwit Bay, WI, near the first recorded bloom events in Lake Superior, and Siskiwit Lake, WI, which is hydrologically connected to Siskiwit Bay via the Siskiwit River (Figure 1.).

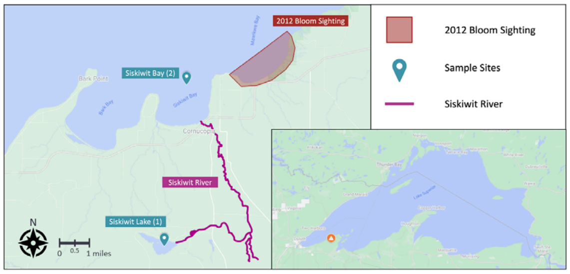

Figure 1. The southern shoreline of Lake Superior at Cornucopia, Wisconsin, USA. Cyan pinpoints and corresponding labels show study areas. The red-shaded area indicates the location of the first recorded cyanobacteria bloom in 2012.

Surface water samples were also collected from a recent 2021 bloom in Siskiwit Lake. Using elemental (%OC and %N) and isotopic indicators ($\delta^{13}C$), the contribution of organic carbon sources over time in the sedimentary deposits of these locations was analyzed. Further, water column particulates including cyanobacteria were collected from a 2021 bloom in Siskiwit Lake to determine the specific pigment makeup of cyanobacterial species in this region.

I. Methods

The objective of this study was to provide historical context for modern-day primary productivity of oligotrophic Lake Superior. To achieve this, the organic carbon sources within the sedimentary record of the southwestern Lake Superior region and the presence of cyanobacteria throughout history were investigated by means of elemental and isotopic analysis. The pigment suite of a modern-day algal bloom was profiled, as well, for further interpretation of the ecological contribution of phytoplankton communities within this region of the watershed.

A. Elemental Analyzer-Isotope Ratio Mass Spectrometry

Each sediment sample was analyzed for its elemental composition (%OC and %N) and stable isotopes ($\delta^{13}C$) at 1-cm resolution using the EA-IRMS instrument at the Large Lakes Observatory (LLO) of the University of Minnesota in Duluth. Caffeine (0.25 mg), Acetanilide (0.40 mg), B-2153 (25.0 mg), and B-2159 (0.50 mg) standards were utilized between every ten sample runs to ensure precision of the instrument and correct for drift. Percent organic carbon, nitrogen, and isotopic data were selected as proxies for the characterization of terrestrial versus aquatic OM within the study site sedimentary record. As was conducted in a review by Meyers (1994), carbon to nitrogen ratios were correlated with $\delta^{13}C$ values for both sites to further distinguish the deposited OM as lacustrine algae, marine algae, C3 land plants, or C4 land plants. Detection of organic carbon was improved by acid (12.0 M HCl) fumigation of carbonate contaminants prior to analysis.

B. High-Performance-Liquid-Chromatography

Water column samples were also taken from Siskiwit Lake during an algal bloom in June of 2021, using porous micron filters. 46 surface water filter samples were freeze-dried after collection and stored in aluminum foil at -20°C. The samples were combined and homogenized into scintillation vials according to collection site as follows: Filter #1) Siskiwit Lake Boat Launch (SLBL), Filter #2) Siskiwit Lake Campground A (SLCA), Filter #3) Siskiwit Lake Campground B (SLCB), Filter #4) Siskiwit Lake Campground, Boat Launch, and Dock, Filter #5) Mixed Locations, Filter #6) Negative Difference (which showed loss in mass). Each homogenized sample was then mixed with approximately 3 mL acetone. The resulting solutions were centrifuged for 5 minutes, sonicated for 1 minute in ice water to free the pigments from the cell membranes, syringe-filtered, and dried using nitrogen gas (Bianchi & Findlay, 1991; Chen et al., 2001; Schreiner et al., 2013; Dr. Michael Shields, Texas A&M University). Finally, the samples were prepared for injection into the HPLC by resolving in 100 µL of acetone, with 100 µL of trans-*β*-Apo-8'-carotenol internal standard (Dr. Michael Shields, Texas A&M University).

The HPLC analysis procedure was adapted from Dr. Michael Shields, Texas A&M University. Using a Dionex Ultimate 3000 for liquid chromatography with a Dionex™ DAD 3000 detector (Chemistry and Biochemistry Department, University of Minnesota Duluth), the absorbance of the injected photosynthetic pigments was measured. Chromatographic separation of each sample was run for approximately 11 minutes, employing a linear gradient from 5% 70:30 (v:v) methanol and tetrabutylammonium acetate (TBAA) to 95% Optima-grade Methanol (Van Heukelem & Thomas, 2001), followed by a 4 minute isocratic hold and 8 minute equilibration with Optima-grade Methanol.

Injections were run through an Acquity UPLC® VanGuard™ Pre-Column (BEH C8 1.7 µm, 2.1 x 5 mm) and Zorbax Rapid Resolution High Definition Eclipse Plus C8 analytical column (95Å, 2.1 x 100 mm, 1.8 µm, Agilent Technologies), maintained at 60°C with a 0.300-mL/min flow rate (Van Heukelem & Thomas, 2001). Excitation and emission wavelengths were set at 450 nm (4-nm bandwidth) and 666 nm (4-nm bandwidth), respectively, with DAD collection at a rate of 20 Hz. The detector's linear range relevant to sample concentrations was evaluated by conducting calibration curves of mixed and individual pigment standards, acquired from DHI Water Quality Institute (Horsholm, Denmark), prior to the sample analysis. The absorption profiles of pigment standards were collected in advance to determine elution order for the carotenoids, chlorophylls, and degradation products of interest:

a) Alloxanthin (0.948 mg/L)
b) Aphanizophyll (0.845 mg/L)
c) β-Carotene (0.832 mg/L)
d) Canthaxanthin (1.648 mg/L)
e) Chlorophyll-*a* (1.707 mg/L)
f) Chlorophyll-*b* (1.190 mg/L)
g) Diadinoxanthin (0.690 mg/L)
h) Diatoxanthin (0.641 mg/L)
i) Echinenone (0.923 mg/L)
j) Fucoxanthin (1.490 mg/L)
k) Myxoxanthophyll (0.764 mg/L)
l) Neoxanthin (0.751 mg/L)
m) Pheophytin-*a* (3.208 mg/L)
n) Violaxanthin (1.118 mg/L)
o) Zeaxanthin (0.782 mg/L)

III. Results

A. Geochemical Evidence of Historic Cyanobacteria Organic Carbon Sources

Total organic carbon content of the sediment cores was analyzed to obtain a relative percentage of OM deposited in the study sites over time (Fig. 2). Sections of the core correspond to depositional time, with the surface of the core (0 cm) correlating to more recent years (Fig. 2). TOC in Siskiwit Lake ranged from 16.94% to 22.02% downcore, with a mean of 19.36% ± 0.04% (Fig. 2).

In Siskiwit Bay, increased sand deposition from 55.5 to 63.5 cm manipulated bulk elemental and isotopic signatures. This distinct layer of sand within the core was posited to have artificially lowered TOC, as sand poorly preserves organic matter (Yeasmin et al., 2017). Increasing TOC from ~50 cm to 0 cm could reflect increased loading of OM from primary producers within the bay. TOC values in the bay were lower overall compared to Siskiwit Lake, which further emphasizes a rising trend in concentration closer to the present day. Such could be indicative of rising productivity of cyanobacteria in Lake Superior, shown by previous work (O'Beirne et al., 2017). While higher inputs of terrestrial OM due to high magnitude storm events could also contribute to higher signals, the lake's high surface area to watershed ratio mediates this contribution.

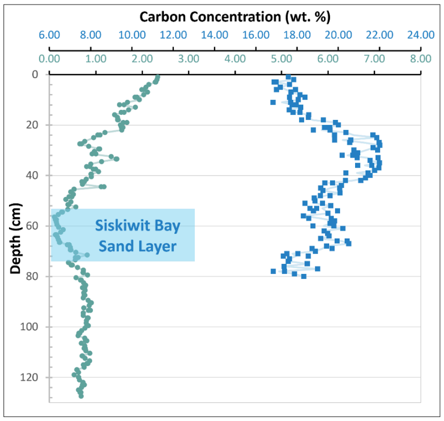

Figure 2. Total organic carbon content (percent weight) of Siskiwit Lake (~80 cm core) and Siskiwit Bay (~127 cm core). TOC of Siskiwit Bay (in green) is plotted on the primary x-axis from 0.00 to 8.00 (wt.%), and Siskiwit Lake TOC (in blue) is plotted on the secondary axis from 6.00 to 24.0 (wt.%). Sediment core resolution in centimeters is oriented on the y-axis by decreasing depth.

Results shown in Figures 3. and 4. exhibited δ^{13}C signatures that identified lacustrine OM, with C:N ratios indicative of algal and C3 land plant sources. δ^{13}C values of -27.89‰ to -24.78‰ (mean of -25.84‰ ± 0.2‰) in Siskiwit Bay reflected higher autochthonous sourcing than Siskiwit Lake (Fig. 3.). As observed in the TOC results, the effect of the sand layer was evidenced in significantly lower isotopic composition from 55.5 to 63.5 cm in the bay. A less enriched isotopic average of -29.0‰ ± 0.1‰ in Siskiwit Lake implied slightly higher allochthonous contribution of OM (Fig. 3.). This could be attributed to a higher ratio of the lake water to surrounding watershed area and more direct interactions between them, introducing terrestrial OM to the lake in high concentrations relative to its size, compared to Siskiwit Bay.

The cross-plot of the elemental and isotopic data shown in Figure 4. was adapted from Meyers (1994) and further used to model the trends of this freshwater algae OM with depth (Figures 4a. and 4b.). Isotopic signatures from 55.5 to 63.5 cm in Siskiwit Bay were included but the C:N ratios were not. Siskiwit Lake exhibited a more consistent C:N than Siskiwit Bay over time (Fig. 4b.), as was expected due to the regular frequency of blooms in the lake and watershed to lake surface area ratio, and higher overall C:N signature. Increasing TOC in Siskiwit Bay was reflected in an increased C:N ratio close to the core surface (Fig. 4a.). C:N ratios in the bay were less consistent over time, where ratios decreased slightly from the bottom to middle of the core and increased from the middle to the surface (Fig. 4a.). As highlighted in Figure 2., C:N trends plotted against lacustrine algae isotope signatures could suggest increasing primary productivity within the bay (Fig. 4.; O'Bierne et al., 2017, Meyers et al., 1994).

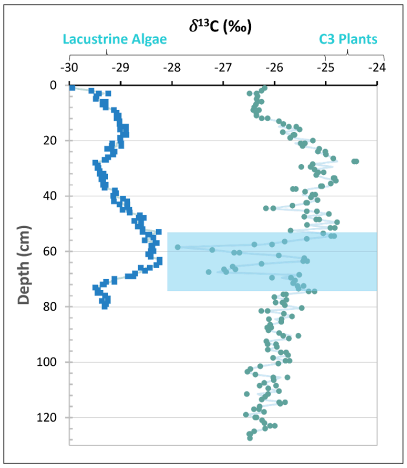

Figure 3. Stable isotope content (per mille) of Siskiwit Bay and Siskiwit Lake. $\delta^{13}C$ ‰ ranges of Siskiwit Bay (in green) and Siskiwit Lake (in blue) are plotted on the x-axis. Sediment core resolution in centimeters is oriented on the y-axis by decreasing depth. Siskiwit Lake reported $\delta^{13}C$ values ranging from -29.94‰ to -28.24‰, with a mean of -29.0‰ ± 0.1‰. δ13C ranges from -27.89‰ to -24.78‰, with a mean of -25.84‰ ± 0.2‰ in Siskiwit Bay. The Siskiwit Bay core sand layer is highlighted in blue.

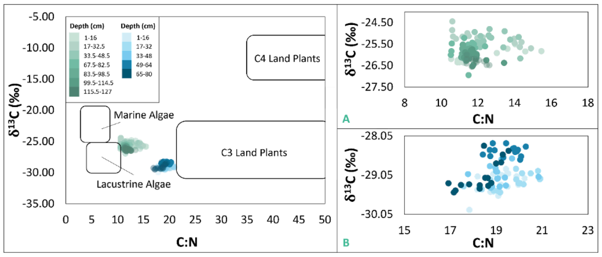

Figure 4. C:N (mol to mol) ratios correlated with $\delta^{13}C$ (‰) to identify primary sources of organic material in the core locations. The bulk identifier graph is adapted from Meyers (1994). C:N ratios are designated by the x-axis, with $\delta^{13}C$ (‰) signatures on the y-axis. Siskiwit Lake points are shown in blue, while Siskiwit Bay points are in green. Sediment core depths are represented by color hue, where darker shades indicate increasing depth (cm). Figures 4a. and 4b. show finer resolution profiles of Siskiwit Bay and Siskiwit Lake, respectively. Isotopic signatures from 55.5 to 63.5 cm in Siskiwit Bay were included but the C:N ratios were not.

B. Pigments Indicative of Cyanobacterial Presence in 2021 Algal Bloom

Results from the water filter pigment extractions showed biomass concentrations predominating from cyanobacteria and chlorophyte sources within the water column during the 2021 Siskiwit Lake bloom. The most abundant pigments, pheophytin-*a* (3.484 mg/gOC) and Chl-*a* (2.991 mg/gOC) (Table 1.), are considered indicators of productivity levels (Bianchi & Canuel, 2011; Huisman et al., 2018; Jeffery & Hallegraeff, 1987). Combined with *β*-carotene (0.618 mg/gOC) (Table 1.), total productivity pigments accounted for 71.41% of bloom biomass (Fig. 6). Cyanobacteria pigments (aphanizophyll, canthaxanthin, echinenone, and myxoxanthophyll) constituted 16.44% of the total pigment biomass, notably higher than the 9.54% (Chl-*b*, neoxanthin, and violaxanthin) contributed by green algae/chlorophytes (Fig. 6; Bianchi & Canuel, 2011; Egeland, 2016; Huisman et al., 2018; Paerl, 2017). Diatoms and/or dinoflagellates and cryptophytes provided 1.70% and 0.91%, respectively (Fig. 6; Bianchi & Canuel, 2011; Egeland, 2016).

IV. Discussion

Elemental and isotopic data obtained from the Siskiwit Bay and Siskiwit Lake sediment deposits exhibited dominant signals of aquatic and terrestrial OM sources, respectively. Increasing trends of organic carbon concentrations were observed in the bay, withholding the artificially lowered TOC content of sand layer, while a fluctuating deposition of OM was found within Siskiwit Lake (Fig. 2). Isotopic carbon signatures were predictably low, or less enriched, overall and shifted towards a less enriched profile approaching present day in both sites (Fig. 3). Separately, before the recent decrease observed from approximately 30 cm to 0 cm, trends in Siskiwit Bay exhibited a general enrichment over time with distinct drops that could be explained by historic cyanobacteria blooms (Fig. 3). With concurrent trends in carbon ratios, the change towards less negative isotope signatures could depict increased algal derived sourcing (Meyers, 1994). Despite remaining near the algal range of 4 to 10, substantial C:N increases approaching the lakebed's surface might posit rising contribution from C3 lands plants and/or less degraded sedimentary OM (Fig. 4a.; Meyers, 1994).

Table 1. Average individual photopigment contributions in 2021 Siskiwit Lake bloom. Concentrations are listed in mg/gOC, along with their percentage of the total biomass present (%) and source type.

Pigment	**Concentration** (mg/gOC)	**Percent of Total Biomass** (%)	**Source** (with references)
Aphanizophyll	0.255	2.57	*Cyanobacteria*[9]
Canthaxanthin	0.100	1.01	*Cyanobacteria*[6,9,19]
Echinenone	1.026	10.34	*Cyanobacteria*[3,6,9]
Myxoxanthophyll	0.106	1.06	*Cyanobacteria*[6,8,9,19]
Zeaxanthin	0.146	1.47	*Cyanobacteria; Higher Plants*[3,6,9]
Fucoxanthin	0.131	1.32	*Diatoms; Dinoflagellates; Brown seaweed*[3,6]
Diadinoxanthin	0.025	0.25	*Diatoms; Dinoflagellates*[3,6]
Diatoxanthin	0.012	0.12	*Diatoms; Dinoflagellates*[6]
Chlorophyll-*b*	0.618	6.22	*Chlorophyta*[3]
Neoxanthin	2.991	30.11	*Chlorophyta; Higher plants*[3,6]
Violaxanthin	3.484	35.08	*Chlorophyta*[6]
Alloxanthin	0.090	0.91	*Cryptophytes*[3]
β-Carotene	0.184	1.85	*All plants; Cyanobacteria; Diatoms*[3]
Chlorophyll-*a*	0.160	1.61	*Photosynthetic plants/microalgae; Diatoms* [9,12]
Pheophytin-*a*	0.605	6.09	*Degradation product of Chl- a; Zooplankton feces*[3]

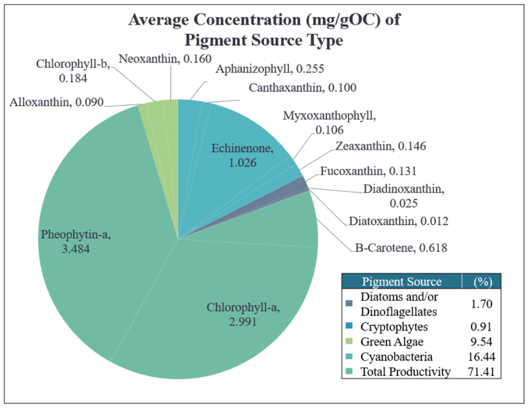

Figure 5. Average pigment concentrations (mg/gOC) in water surface samples from Siskiwit Lake 2021 bloom. Pigments are grouped according to general source type, with diatoms and/or dinoflagellates in navy, cryptophytes in blue, green algae in lime, cyanobacteria in teal, and total productivity markers in sage. Percent composition of total organic biomass (%) per source type is shown in the lower left hand corner table, which was grouped according to Table 1.

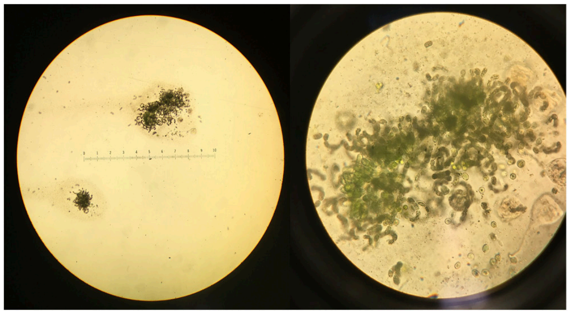

Figure 6. Microscopy images of microalgae samples taken from the surface water of the 2021 Siskiwit Lake bloom. Microscopic examination suggested the samples belonged to the Dolichospermum genus; likely D circinale or D lemmermannii, the latter of which has previously been identified in Lake Superior blooms. Images were kindly provided by Kaela Natwora and Cody Sheik of the Large Lakes Observatory.

Siskiwit Lake bulk elemental and isotopic signatures were expected to reveal sedimentary OM largely derived from algal sources, based on the lake's higher frequency of cyanobacteria blooms. While isotopic signatures alone could be interpreted as supporting this hypothesis (Fig 3.), relatively sustained C:N ratios downcore revealed higher inputs of carbon-rich material than nitrogen-rich materials, likely contributed by C3 plants in the surrounding watershed (Fig. 4.).

When reviewed alongside the characterization of Siskiwit Lake, interpretations of higher autochthonous sourcing in Lake Superior were supported by increasing TOC and $\delta^{13}C$ values and lower C:N ratios. Elevated C:N ratios near the lakebed surface do not completely denounce but marginally lower confidence in these conclusions. Further differentiating whether increasing elemental ratios are truly due to increased terrestrial input, such as vascular land plants being deposited in the sediment through high magnitude discharge events, or higher abundance of autochthonous algal sources could be sought in historical environmental records. Moreover, further statistical modelling to distinguish these endmembers and cross-analysis of downcore phytoplankton pigment profiles would provide more confidence in bulk OM proxy interpretations.

While data for the downcore pigments in Siskiwit Bay and Siskiwit Lake were not yet available, pigment identification and quantification of the 2021 Siskiwit Lake algal bloom showed dominance of cyanobacteria and green algae. The unique pigment suite also indicated the presence of diatom, dinoflagellate, cryptophytes, general photosynthetic pigments present in several species, and other degradation products (Table 1.). High concentrations of chlorophyll-*a* suggested high primary productivity overall (Fig. 5), as expected. Equally high concentrations of pheophytin-*a* were also expected and likely originated from bacterial decay or grazing activities (Fig. 5) (Leavitt, 1993). Considerable cyanobacteria and chlorophyte sourcing was implied, with lower contribution from diatom, dinoflagellate, and other cryptophyte phytoplankton communities (Fig. 5). While toxicity has not been assessed, these data suggested the presence of potentially nuisance cyanobacteria species present in Siskiwit Lake, WI, and that these inland lake blooms could be potential drivers of cyanobacteria accumulation in Lake Superior via fluvial seeding (i.e. Siskiwit River) (Fig. 1; Sterner et al., 2020). The data would also corroborate the presence of cyanobacteria paleo-indicators in the sediment cores, providing a modern-day reference suite for ancient algal communities.

V. Contextualization of Work

The recent rise of cyanobacterial blooms in Lake Superior influences much more than the health of the immediate aquatic ecosystem. Not only one of the largest freshwater lakes but also one of the cleanest, Lake Superior supplies important environmental services, particularly potable water, affecting an expansive population (Sterner et al., 2020). One of many projects examining the lake's health at the Large Lakes Observatory (LLO), this study contributes a cornerstone to improving our understanding of climate change interactions within Lake Superior's watershed. An initial profiling of the lake's sedimentary record and current bloom biomass composition provides temporal insight into the region's cyanobacteria communities. It could also reveal spatial connections to inland lakes and rivers with the hydrological potential to seed blooms along the southern shores. In further research through the LLO, this preliminary data will be paired with the presence of algal pigments within the sedimentary record to reconstruct intervals of high productivity and identify potential bloom periods within the Lake Superior region. Continued work may include genomic sequencing and species identification, providing the structure for HAB prediction models and water quality management strategies.

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