

Dissolved organic matter structure and quality across a gradient of north temperate lakes

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Meredith Kadjeski
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Abstract

Water chemistry of ten north temperate lakes ranging in dissolved organic matter (DOM) levels was analyzed in this study. A number of biological, chemical, and physical variables were measured in each lake to characterize lake conditions and to use as predictors to explain the variation in DOM size and structure among lakes. Analysis of ultraviolet-visible absorption spectra contained information on whether the organic matter is produced in the water body or in its catchment. Various spectral indices, which are widely used in aquatic studies to determine the origin and composition of organic matter, were applied. In particular, the slope of the 275-295 nm and 350-400 nm region and the ratio of these slopes were related to DOM molecular weight. The use of spectral indices offers valuable information in the characterization of organic matter found in dissimilar water types.

Introduction

Dissolved organic matter (DOM) consists of organic molecules of varied origin and composition found within aquatic systems, which influences water quality and fuels microbial metabolism, but its origins, bioavailability, and chemical composition are poorly understood. DOM is also an important component in the functioning of aquatic ecosystems, as it can serve as a primary food source for aquatic food webs as well as influencing light availability and water temperature. The properties of DOM are diverse and depend on its source (Helms et al. 2008). Organic matter can come from aquatic (e.g. phytoplankton) or terrestrial (e.g. decomposed plant material) sources. The relative contribution of these sources to total DOM depends on the body of water, though in many lakes a considerable part is derived from terrestrial sources (Wilkinson et al. 2013).

The size and structure of DOM can influence how bioavailable the organic matter is for microbes and other constituents of the food web. The bioavailability of DOM could also influence carbon cycling. Lakes with less labile DOM may store more carbon in the sediment (though even lakes with low organic matter content can store a large amount of carbon) or export more downstream whereas lakes with more labile DOM may emit more carbon to the atmosphere (Tranvik et al. 2009). Organic matter, particularly the humic fraction of dissolved organic carbon, can also affect light penetration in aquatic ecosystems (Cuthbert and del Giorgio 1992) and protect organisms from UV radiation (Helms et al. 2008). Humic compounds are products of plant decomposition that affect aquatic organisms, which is important for the ecosystem's phototrophs that need light (Cuthbert and del Giorgio 1992; Ask et al. 2012). In addition to reducing light energy transmission through water (Kirk 1976), these decomposed products also affect the

solubility, transport, and bioavailability of nutrients and contaminants (Mierle and Ingram 1999; Peterson 1991).

Most studies concerning DOM in lakes both at UNDERC and elsewhere have measured total DOM concentration, however the chemical qualities of DOM, such as size and structure, are less frequently studied. The molecular composition of lake DOM is highly variable and dynamic, particularly reflecting the source of the organic matter (Kothawala et al. 2014). Further analysis of the DOM of aquatic ecosystems is crucial for better understanding the influence of the terrestrial surroundings on lake ecosystem function. There are a number of analytical techniques such as fluorescence spectroscopy (Hudson et al. 2007; Kothawala et al. 2014) and stable isotopes (Wilkinson et al. 2013) that can be used to identify the source and characteristics of DOM. The spectral properties of DOM, such as the absorbance curve from 275-295 nm, can also provide valuable information about the size and structure of DOM (McKnight et al. 2011; Coble 2007; Helms et al. 2008; Chen et al. 2011; Garcia et al. 2015). Similar to stable isotopes, DOM absorbance curves could function as a signature of the source material of the organic matter.

This study seeks to characterize the size, structure, and source of DOM, in numerous lakes to better understand the source and influence of DOM on these lake ecosystems. The study lakes are characterized by different concentrations of colored dissolved organic carbon, forming a gradient ranging from clear-water to brown-water lakes. The aim of this study is to link the spectral characteristics of lake water DOM to lake chemistry and DOM size and structure, with the intent to understand the source of DOM in UNDERC lakes.

Methods

Study area

The ten study lakes (Figure 1, Table 1), located in the University of Notre Dame Environmental Research Center (UNDERC) in Gogebic County, Michigan, were sampled four times over a period of 50 days between the end of May and mid-July, 2016. The selected lakes at UNDERC span a gradient of low to high dissolved organic matter (DOM), ranging from ‘clear’ to ‘brown’ to dystrophic. The brown color of dystrophic lakes is the result of high concentrations of humic substances and organic acids suspended in the water. This region consists of flat terrain with extensive areas of bog or low-lying forest between the numerous glacial kettle lakes.

Sample collection and analysis

The studied lakes are highly variable with respect to environmental variables; a number of biological, chemical, and physical variables were measured in each lake to characterize lake conditions and to use as predictors to explain the variation in DOM size and structure among lakes. Water samples were collected from surface waters (at a depth of 0.5 m) from a central, deep spot in each lake (identified using bathymetric maps) and were analyzed for pH, chlorophyll-*a* concentration, and spectral properties. To measure pH, the probe was placed in sample water for 15 minutes before being inserted into an airtight BOD bottle filled with sample water for five minutes, and repeated once. For chlorophyll, two replicates of 200 mL of well-shaken sample water was filtered through a 47 mm GF/F filter. Filter towers and filters were rinsed with deionized water. Dissolved oxygen (DO) and temperature profiles were measured in each lake using an optical probe (YSI ProODO). DO and temperature readings were taken at 0.5

m intervals from 0-7 m, then at 1 m intervals to 0.0 mg/l of oxygen. In addition, the attenuation of light in the water column was measured using a Li-Cor submersible light meter. Both scalar (underwater) and cosine (surface) sensors were used to determine the scale of light at depth. The sensor was lowered from the sunny side of the boat; readings were taken at the surface and at 0.25 m intervals to 1.0 m, then at 0.5 m intervals to approximately 1% surface irradiance. The light attenuation was calculated as the slope of the linear regression $\ln(I_z/I_0) = -k \cdot z$ (where I_z is light intensity at depth z , I_0 is light intensity at the surface, k is the light extinction coefficient, and z is depth below the surface). Gravimetric zooplankton biomass (dry weight) was also measured to determine if levels of DOM quality correlate with aquatic consumer biomass. A 153 micron Wisconsin plankton net was lowered to approximately two-thirds of the depth of each lake and retrieved at a constant rate of three seconds per meter. Two vertical zooplankton tows were taken and pooled into a single 120 mL jar.

Spectral corrections and S determination

Though color can be measured by visual comparison of water samples, the spectrophotometric determination of color was used to characterize DOM size and structure (Cuthbert and Del Giorgio 1992). The absorption spectra from filtered water samples (25 mm Whatman GF/F Glass Microfiber Filter, 0.6 – 0.8 μm nominal pore size) were obtained in a UV-visible spectrophotometer, using a 1 or 10 cm quartz cuvette. To extract information about DOM properties from these spectra, several spectral parameters were calculated. Absorbance values were converted to absorption coefficients as follows: $a = 2.303 (\text{Abs}/r)$, where a = absorption coefficient (m^{-1}), Abs = absorbance and r = path length (m). The most frequently used wavelength to define an index of terrestrial input is 440 nm (Cuthbert and del Giorgio 1992). The

coefficient a_{440} is indicative of water color and is associated with allochthonous DOM inputs (Rasmussen et al. 1989). A_{254} and a_{350} have also been used as a measure of aromatic constituents (Martin-Mousset et al. 1997; McKnight et al. 2001; Weishaar et al. 2003; Spencer et al. 2008) and organic substances (Hernes and Benner 2003; Spencer et al. 2008, 2009), respectively.

Spectral slopes were calculated to provide further insights into the molecular weight of DOM. DOM absorbance was assumed to be zero above 700 nm; therefore, the averaged UV-visible absorbance between 700 and 800 nm was subtracted from each spectrum as a baseline correction (Helms et al. 2008). The spectral slopes for the intervals of 275-295 nm ($S_{275-295}$) and 350-400 nm ($S_{350-400}$) were calculated by regressing the log-transformed spectral data against wavelength and multiplying by -1 (Helms et al. 2008). Slopes are indicative of the molecular weight of the DOM such that the steeper slopes (higher S values) have been interpreted as corresponding to low molecular weight DOM, while shallower slopes (smaller S) indicate a comparatively higher aromatic content and molecular weight (Helms et al. 2008; Spencer et al. 2009; De Laurentiis et al 2012; Fleck et al. 2013). Specifically, $S_{275-295}$ is used to indicate DOM composition and $S_{350-400}$ provides the relative molecular weight and size of DOM (Helms et al. 2008). The ranges 275-295 nm and 350-400 nm were chosen because the spectra indicated that the greatest variations in spectral slopes for the interval of 300-700 nm occur within these narrow bands (Helms et al. 2008). Additionally, the slope ratio (S_R) can also be used as a proxy for molecular weight (Helms et al. 2008); Lower S_R values reflect an increased DOM input from the surrounding forest, runoff, and groundwater (Garcia et al. 2015). S_R was calculated by $S_{275-295} / S_{350-400}$.

Incubation experiments

In order to investigate different potential sources of DOM in the lake, a set of incubation experiments were performed to characterize the size and structure of DOM from different terrestrial and aquatic sources. Four substrates, macrophytes, sphagnum moss, humus (including leaves and other forest-floor materials), and green *Acer saccharum* leaves, representing potential sources of DOM found in the lakes were collected at the beginning of July. Approximately equal masses of each substrate were added to bottles and filled to the top with deionized water. Each substrate was incubated at two temperatures; one replicate of substrates was stored in a dark refrigerator and the second was covered with tin foil and stored at room temperature in a lab hood. Both treatments were kept in the dark for the duration of the incubation. Water samples for spectrophotometric analysis were taken on days 1, 3, and 7, and the spectral slope of the leachate from the organic matter sources was calculated. Spectral qualities of the leachate were compared to lake samples in an attempt to identify the dominant sources of DOM in the lakes.

Data analysis

Changes in spectral slopes ($S_{275-295}$ and $S_{350-400}$) were compared for each lake across the four sampling weeks using. An ANOVA was performed to determine the spectral slope varied significantly among lakes. In addition, post-hoc tests (Tukey's Honest Significant Difference) were performed to confirm where the differences occurred between lakes. Regression analysis was calculated across all lakes and sampling weeks to see what environmental variables (chlorophyll-*a*, k, pH, zooplankton) predict and correlate spectral slope.

Results

Limnological variables

The pH varied between acid and slightly basic in the set of study lakes (Table 1). Lakes Tender Bog, Hummingbird, and Cranberry had a slightly more acidic pH, while lakes Paul, Peter, and Morris had slightly higher pH values. Morris was the only basic lake (7.10 ± 0.14). A wide range in the chlorophyll-*a* concentration was observed among the studied lakes (Table 1). The highest values were recorded for Hummingbird ($33.17 \pm 32.54 \mu\text{g L}^{-1}$), followed by Morris ($11.59 \pm 2.06 \mu\text{g L}^{-1}$). The mean chlorophyll-*a* concentrations were $<6.50 \mu\text{g L}^{-1}$ in the remaining study lakes.

DOM characterization

The absorption scans of the epilimnion lake water showed increasing values across the spectrum from lakes Tender Bog, Hummingbird, Cranberry, and Morris; to East Long and Tuesday; and to Peter, West Long, Paul, and Crampton. The highest absorption values were recorded in the UV region for all lakes, and were variable among lakes. Above 400 nm more similarity among lakes was observed, although Tender Bog had higher absorbance values (Table 2).

The absorbance-based DOM characterization, which included three absorption coefficients, exhibited a range of values (Table 2). The absorption coefficients a_{254} , a_{350} , a_{440} exhibited higher values in lakes Morris, Cranberry, Hummingbird, and Tender Bog, and comparatively lower values in lakes West Long, Peter, Crampton, and Paul.

The lowest spectral slope $S_{275-295}$ was observed for Tender Bog with increasing slopes in Peter, Paul, and Hummingbird lakes. In general, the variability over time within a lake was small (with

the exception of Crampton) compared to the variability in spectral slope between lakes (Figure 2). Similarly, the slope $S_{350-400}$ increased from Hummingbird, moving towards Crampton (Table 2). The slope ratio S_R ($S_{275-295}:S_{350-400}$) was highest for Paul (1.09), though all of the lakes exhibited low values (<2).

A significant negative correlation of $S_{275-295}$ with chlorophyll-*a* concentration, light attenuation, and a_{440} was found (Figure 3a, 3b, 3c), in addition to a significant positive correlation of $S_{275-295}$ with pH (Figure 3d) and zooplankton biomass (Figure 4).

Leachate

Results from the incubation experiments displayed a relatively constant $S_{275-295}$ across the week-long sampling period for each source, with the exception of the macrophytes stored at room temperature (as the absorbance was higher than the equipment could accurately measure, resulting in drastically higher spectral slopes) (Figure 5). Only the samples stored at a lower temperature were compared to the lake samples; the cold temperature decreased microbial activity within the bottles, allowing just the source material to leach as much as possible. The results obtained from the incubation experiments were compared to $S_{275-295}$ values for the lake samples (Figure 6). ANOVA indicated that there were significant differences between lakes ($F = 70.098$, $df = 9$, $p = <0.001$). Post-hoc analysis on compared pairwise combinations of lakes (Figure 6, lakes that share a letter with another lake are therefore not significantly different from each other). Lakes Peter, Paul, and Crampton were all significantly different from the remaining seven lakes (all p -values <0.05); however they were not significantly different from each other (Table 3). Tender Bog, Hummingbird, Cranberry, and Morris are not significantly different from

each other. Additionally, lakes with low absorbance values were located in the range of humus and sphagnum, which were two sources with shallower spectral slopes.

Discussion

Characterization of the DOM of the study lakes highlighted that a combination of spectral parameters provides insight into the origin and molecular weight of the DOM. The lakes, which were characterized via coefficients (a_{254} , a_{350} , and a_{440}), spectral slopes ($S_{275-295}$ and $S_{350-400}$), and the slope ratio (S_R), appeared to be affected by a combination of allochthonous DOM and autochthonous DOM.

Overall, the three absorption coefficients ranged from low to extremely high, whereas Helms et al. (2008) and Garcia et al. (2015) had values ranging from extremely low to low. A_{254} has been used as a measure of the contribution of humic substances rich in aromatic constituents, with higher a_{254} thereby implying a higher aromaticity and suggesting a supply of terrestrial DOM (Martin-Mousset et al. 1997; McKnight et al. 2001; Weishaar et al. 2003; Spencer et al. 2008). The highest values of a_{350} also indicate higher lignin phenol concentrations (organic polymers that form important structural materials in the support tissues of vascular plants and some algae) (Hernes and Benner 2003; Spencer et al. 2008, 2009). The coefficient a_{440} , indicative of water color and associated with allochthonous DOM inputs (Rasmussen et al. 1989), indicated higher values for lakes Morris, Cranberry, Tender Bog, and Hummingbird ($>10.0 \text{ m}^{-1}$), being lower in lakes Crampton, Paul, and Peter ($<2.6 \text{ m}^{-1}$). These results collectively indicated a relatively higher terrestrial input in lakes Morris, Cranberry, Tender Bog, and Hummingbird.

The spectral slope $S_{275-295}$ calculated for all lakes ranged between 13 and $19 \times 10^{-3} \text{ nm}^{-1}$. Similarly, Garcia et al. (2015) reported $S_{275-295}$ values between 15 and $25 \times 10^{-3} \text{ nm}^{-1}$. Overall, the low $S_{275-295}$ values recorded for all of these lakes are indicative of the predominance of high molecular weight material in the DOM. The steeper slopes in lakes Peter, Paul, and Crampton indicate that low molecular weight DOM likely from aquatic sources is present. On the other hand, the smaller $S_{275-295}$ values in lakes Tender Bog and Hummingbird indicate that the higher weight DOM that is often associated with terrestrial sources is present.

Comparison of the spectra obtained from lake samples showed that $S_{275-295}$ is inversely related with chlorophyll-*a* concentrations, *k* (attenuation coefficient), and a_{440} ; thus these characteristics are inversely related to the molecular weight in a water sample. Algae produce low molecular weight DOM, and lower molecular weight DOM is more bioavailable. Light is lost quicker in 'brown lakes' that have higher molecular weight DOM from terrestrial sources. Finally, a_{440} is indicative of terrestrial carbon input. Likewise, Helms et al. (2008) also found that spectral slopes (both $S_{275-295}$ and $S_{350-400}$) were highly correlated to molecular weight. Comparison of the spectra also showed that $S_{275-295}$ is directly related with pH and zooplankton biomass. A lower pH (more acidic waters) suggests a higher terrestrial carbon input (Wilkinson et al. 2013). In addition, lakes with high terrestrial inputs have low zooplankton biomass (Carpenter et al. 2016).

Despite the small set of lake samples included in the present study, these analyses highlight their interactions with the catchment, allowing better understanding of the trophic condition of these

lakes as indicated by their spectral properties and chlorophyll-*a* concentrations. As the condition of lakes fluctuate with their surroundings, with remote lakes being more sensitive to changes in their catchments, sustained environmental assessments may accurately track the impacts of climate and anthropogenic changes on the landscape (Garcia et al. 2015).

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Literature Cited

- Ask, J., Karlsson, J., Jansson, M. 2012. Net ecosystem production in clear-water and brown water lakes. *Global Biogeochemical Cycles* 26, GB1017.
- Carpenter, S.R. Cole, J.J., Pace, M.L., and Wilkinson, G.M. 2016. Response of plankton to nutrients, planktivory and terrestrial organic matter: a model analysis of whole-lake experiments. *Ecology Letters*, 19: 230-239.
- Chen, H., Zheng, B., Song, Y., and Qin, Y. 2011. Correlation between molecular absorption spectral slope ratios and fluorescence humification indices in characterizing CDOM. *Aquatic Sciences* 73: 103-112.
- Coble, P.G. 2007. Marine optical biogeochemistry: the chemistry of ocean color. *Chemical reviews* 107: 402-418.
- Cuthbert, I.D. and del Giorgio, P.1992. Toward a standard method of measuring color in freshwater. *American Society of Limnology and Oceanography, Inc.* 37(6): 1319-1326.

- De Laurentiis, E., Minella, M., Maurino, V., Minero, C., Brigante, M., Mailhot, G., and Vione, D. 2012. Photochemical production of organic matter triplet states in water samples from mountain lakes, located below or above the tree line. *Chemosphere*, 88(10): 1208-1213.
- Fleck, J.A., Gill, G., Bergamaschi, B.A., Kraus, T.E., Downing, B.D., and Alpers, C.N. 2014. Concurrent photolytic degradation of aqueous methylmercury and dissolved organic matter. *Science of the Total Environment*, 484: 263-275.
- Garcia, P.E., Dieguez, M.C., and Queimalinos, C. 2015. Landscape integration of North Patagonian mountain lakes: a first approach using characterization of dissolved organic matter. *Lakes and Reservoirs: Research and Management* 20: 19-32.
- Helms, J.R., Stubbins, A., Ritchie, J.D., and Minor, E.C. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography* 53(3): 955-969.
- Hernes, P.J. and Benner, R. 2003. Photochemical and microbial degradation of dissolved lignin phenols: implications for the fate of terrigenous dissolved organic matter in marine environments. *Journal of Geophysical Research: Oceans*, 108: C9.
- Hudson, N., Baker, A., and Reynolds, D. 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters – A review. *River Research and Applications* 23: 631-649.
- Kirk, J.T.O. 1976. Yellow substance (gelbstof) and its contribution to the attenuation of photosynthetically active radiation in the aquatic environment, p. 369-390. In B. Allard et al. [eds.], *Humic substances in the aquatic and terrestrial environment*. Springer.
- Kothawala, D.N., Stedmon, C.A., Muller, R.A., Weyhenmeyer, G.A., Kohler, S.J., and Tranvik, L.J. 2014. Controls of dissolved organic matter quality: evidence from a large-scale boreal lake survey. *Global Change Biology* 20: 1101-1114.
- Martin-Mousset, B., Croue, J.P., Lefebvre, E., and Legube, B. 1997. Distribution and characterization of dissolved organic matter of surface waters. *Water Research* 31:541-553.
- McKnight, D.M., Boyer, E.W., Westerhoff, P.K., Doran, P.T., Kulbe, T.K., and Andersen, D.T. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography* 46: 38-48.
- Mierle, G. and Ingram, R. 1991. The role of humic substances in the mobilization of mercury from watersheds. *Water Air Soil Polluti.* 56: 349-357.
- Peterson, R.C., Jr. 1991. The contradictory biological behavior of humic substances in the aquatic environment, p. 369-390. In B. Allard et al. [eds.], *Humic substances in the*

aquatic and terrestrial environment. Springer.

- Peuravuori, J and Pihlaka, K. 1997. Isolation and characterization of natural organic matter from lake water: comparison of isolation with solid adsorption and tangential membrane filtration. *Environmental International*, 23(4): 441-451.
- Rasmussen, J.B., Godbout, L. and Schallenberg, M. 1989. The humic content of lake water and watershed and lake morphometry. *Limnology and Oceanography*, 34: 1336-1343.
- Spencer, R.G.M., Aiken, G.R., Butler, K.D., Dornblaser, M.M., Striegl, R.G., and Hernes, P.J. 2009. Utilizing chromophoric dissolved organic matter measurements to derive export and reactivity of dissolved organic carbon exported to the Arctic Ocean: a case study of the Yukon River, Alaska. *Geophysical Research Letters*, 36(6).
- Spencer, R.G.M. Aiken, G.R., Wickland, K.P., Striegl, R.G., and Hernes, P.J. 2008. Seasonal and spatial variability in dissolved organic matter quantity and composition from the Yukon River basin, Alaska. *Global Biogeochemical Cycles*, 22(4).
- Summers, R.S., Cornel, P.K., and Roberts, P.V. 1987. Molecular size distribution and spectroscopic characterization of humic substances. *Science of the Total Environment* 62: 27-37.
- Tranvik, L.J., Downing, J.A., Cotner, J.B., Loiselle, S.A., Striegl, R.G., Ballatore, T.J., Dillion, P., Finlay, K., Fortino, K., Knoll, L.B., Kortelainen, P.L., Kutser, T., Larsen, S., Laurion, I., Leech, D.M., McCallister, S.L., McKnight, D.M., Melack, J.M., Overholt, E., Porter, J.A., Prairie, Y., Renwick, W.H., Roland, F., Sherman, B.S., Schindler, D.W., Sobek, S., Tremblay, A., Vanni, M.J., Verschoor, A.M., von Wachenfeldt, E., and Weyhenmeyer, G.A. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography* 54(6, part 2): 2298-2314.
- Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., and Mopper, K. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental science & technology*, 37(20): 4702-4708.
- Wilkinson, G.M., Pace, M.L., and Cole, J.J. 2013. Terrestrial dominance of organic matter in north temperate lakes. *Global Biogeochemical Cycles* 27: 1-9.

Figures and Tables

Table 1. Morphological, chemical, and biological characteristics of study lakes at UNDERC. SA (lake surface area), maximum depth, and TP (total phosphorus) data provided by Wilkinson et al. (2013).

Lake Name	SA (ha)	Depth (m)	pH	TP ($\mu\text{g L}^{-1}$)	Chl- <i>a</i> ($\mu\text{g L}^{-1}$)
Crampton	25.2	15.2	5.95 \pm 0.27	26.5	2.25 \pm 0.42

Cranberry	2.9	7.3	4.39 ± 0.11	31.1	6.36 ± 2.28
East Long	No data	No data	5.83 ± 0.30	No data	5.01 ± 1.84
Hummingbird	1.1	7.6	4.75 ± 0.09	49.0	33.17 ± 32.54
Morris	5.8	6.7	7.10 ± 0.14	66.4	11.59 ± 2.06
Paul	1.5	12.0	6.44 ± 0.28	28.0	2.03 ± 0.88
Peter	2.4	18.0	6.48 ± 0.28	20.3	3.47 ± 1.86
Tender Bog	0.4	10.1	4.16 ± 0.09	35.1	3.74 ± 1.40
Tuesday	2.4	15.0	5.79 ± 0.16	32.2	4.18 ± 0.51
West Long	3.6	14.0	6.14 ± 0.29	41.9	4.05 ± 1.90

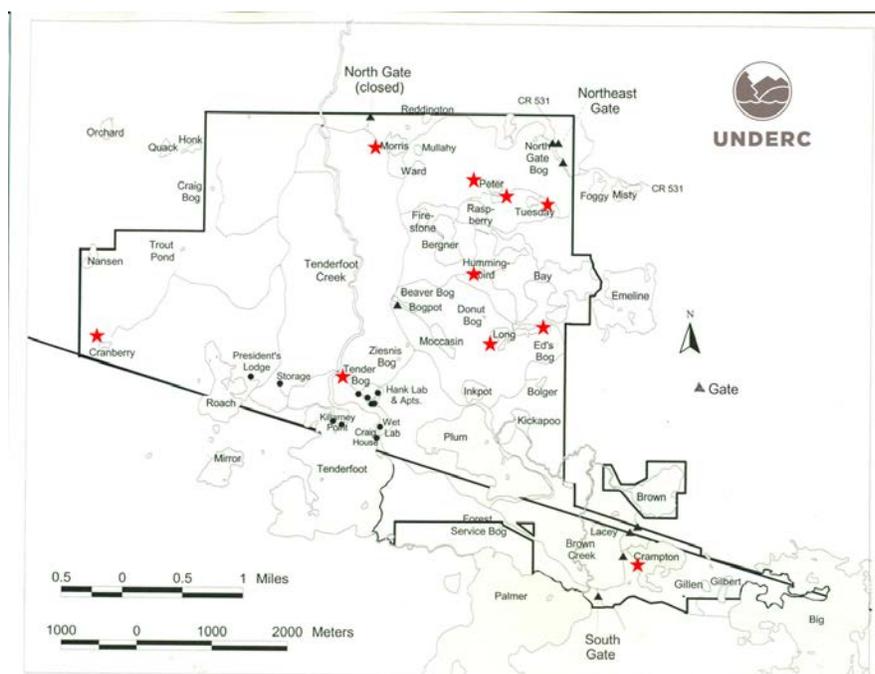


Figure 1. Map of study area and ten north temperate lakes at UNDERC.

Table 2. Spectral parameters measured for each sample from the ten study lakes.

Lake Name	DOY	(nm ⁻¹) S ₂₇₅₋₂₉₅	(nm ⁻¹) S ₃₅₀₋₄₀₀	(nm ⁻¹) S _R	(m ⁻¹) a ₂₅₄	(m ⁻¹) a ₃₅₀	(m ⁻¹) a ₄₄₀
Crampton	145	0.0187	0.0180	1.0431	21.67	No data	1.17
Crampton	156	0.0169	0.0164	1.0335	27.47	6.75	1.77
Crampton	179	0.0164	0.0163	1.0024	32.15	8.13	2.05
Crampton	193	0.0182	0.0179	1.0186	25.72	5.80	1.36
Cranberry	148	0.0139	0.0176	0.7921	184.70	51.82	11.98
Cranberry	155	0.0138	0.0177	0.7797	198.06	55.96	13.13
Cranberry	180	0.0137	0.0176	0.7790	195.99	55.73	12.90
Cranberry	194	0.0138	0.0173	0.7961	182.86	51.82	12.21
East Long	147	0.0146	0.0167	0.8773	89.82	25.10	6.22

East Long	159	0.0142	0.0161	0.8800	105.02	30.86	7.60
East Long	180	0.0143	0.0165	0.8675	97.19	27.41	6.68
East Long	194	0.0148	0.0164	0.9038	94.65	26.48	6.68
Hummingbird	148	0.0134	0.0164	0.8140	226.38	67.71	17.04
Hummingbird	155	0.0133	0.0163	0.8145	273.83	82.45	20.73
Hummingbird	181	0.0132	0.0162	0.8163	289.03	87.05	22.57
Hummingbird	193	0.0132	0.0160	0.8263	288.34	87.28	23.03
Morris	145	0.0141	0.0160	0.8802	150.62	42.14	10.82
Morris	156	0.0135	0.0163	0.8323	177.56	51.13	13.13
Morris	179	0.0139	0.0162	0.8577	180.32	51.36	12.67
Morris	194	0.0143	0.0165	0.8671	167.89	46.52	11.05
Paul	146	0.0179	0.0167	1.0696	21.86	5.18	1.43
Paul	158	0.0174	0.0171	1.0182	24.23	5.94	1.68
Paul	183	0.0177	0.0170	1.0375	24.39	5.99	1.73
Paul	194	0.0177	0.0162	1.0919	24.64	5.96	1.68
Peter	146	0.0182	0.0176	1.0341	32.63	7.55	1.84
Peter	158	0.0171	0.0168	1.0166	36.76	8.84	2.28
Peter	183	0.0171	0.0166	1.0295	39.84	9.79	2.51
Peter	194	0.0173	0.0164	1.0547	38.07	9.30	2.44
Tender Bog	148	0.0133	0.0170	0.7803	236.06	68.17	14.97
Tender Bog	155	0.0130	0.0176	0.7425	359.27	105.25	23.03
Tender Bog	179	0.0132	0.0173	0.7588	385.98	114.00	25.33
Tender Bog	194	0.0132	0.0172	0.7663	358.81	106.17	24.41
Tuesday	146	0.0156	0.0167	0.9348	79.45	20.50	4.84
Tuesday	159	0.0155	0.0174	0.8925	80.14	20.27	4.61
Tuesday	181	0.0153	0.0178	0.8635	82.22	20.96	4.61
Tuesday	194	0.0150	0.0170	0.8862	92.58	24.64	5.76
West Long	147	0.0160	0.0166	0.9652	37.40	6.75	1.98
West Long	159	0.0160	0.0164	0.9757	48.71	12.39	3.09
West Long	180	0.0156	0.0166	0.9441	53.64	14.19	3.82
West Long	194	0.0157	0.0162	0.9662	52.37	13.73	3.59

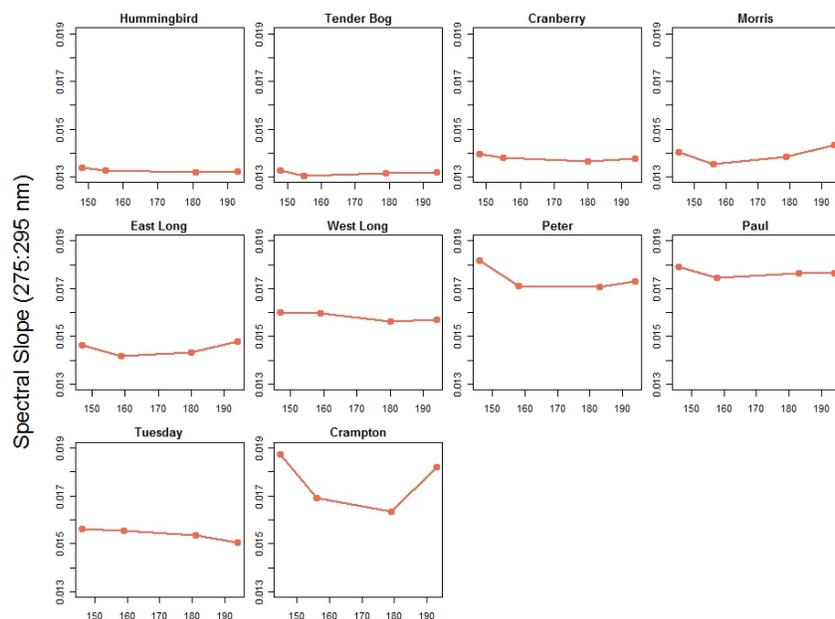


Figure 2. Time series of $S_{275-295}$ in each lake throughout the sampling period.

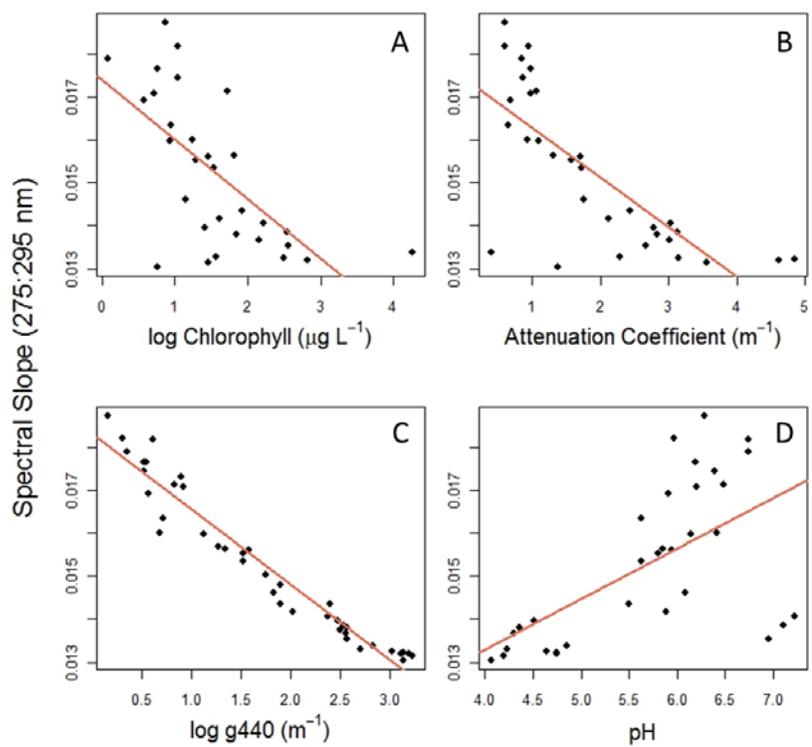


Figure 3. Regression plot of $S_{275-295}$ and chlorophyll-*a* concentration (A) ($y = -0.0014x + 0.0174$; $R^2 = 0.4282$, $F = 20.97$, $df = 26$, p -value <0.001), attenuation coefficient (B) ($y = -0.0012x + 0.0174$; $R^2 = 0.5635$, $F = 38.72$, $df = 30$, p -value <0.001), a_{440} (C) ($y = -1744e^3x + 1.83e^2$; $R^2 = 0.9433$, $F = 632.8$, $df = 38$, $p <0.001$), and pH (D) ($y = 0.0012x + 0.0086$; $R^2 = 0.3613$, $F = 16.97$, $df = 30$, p -value <0.001).

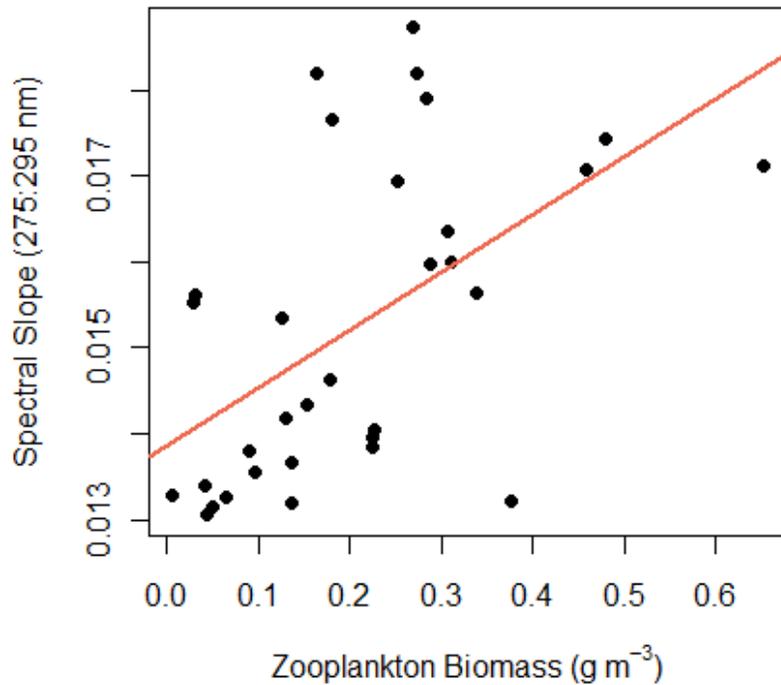


Figure 4. Regression plot of $S_{275-295}$ and zooplankton biomass ($y = 0.0067x + 0.0139$; $R^2 = 0.2987$, $F = 12.78$, $df = 30$, p -value = 0.001212).

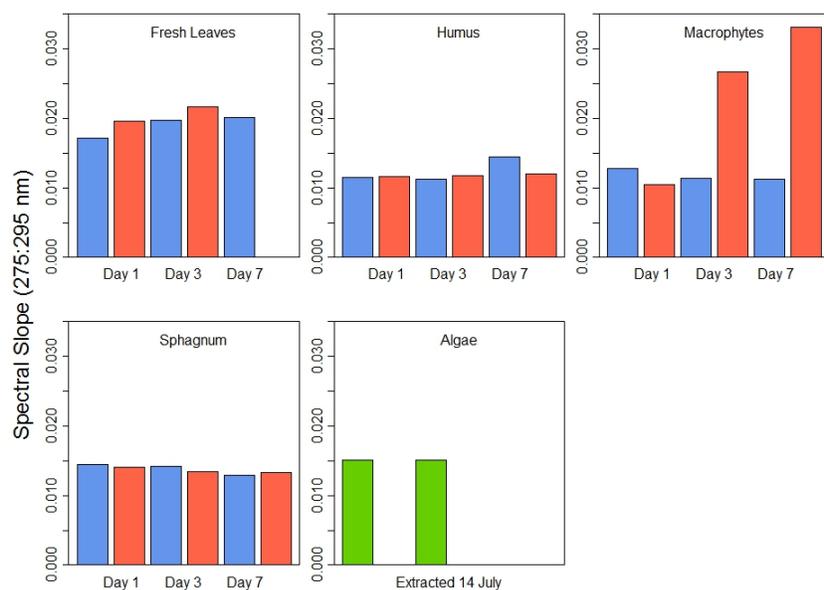


Figure 5. Barplots displaying changes in $S_{275-295}$ over seven day incubation experiment. Blue bars are those substrates stored in a dark refrigerator, and red bars are those substrates stored at room temperature in a lab hood. Two algae samples were extracted after the incubation experiment as another source for comparison.

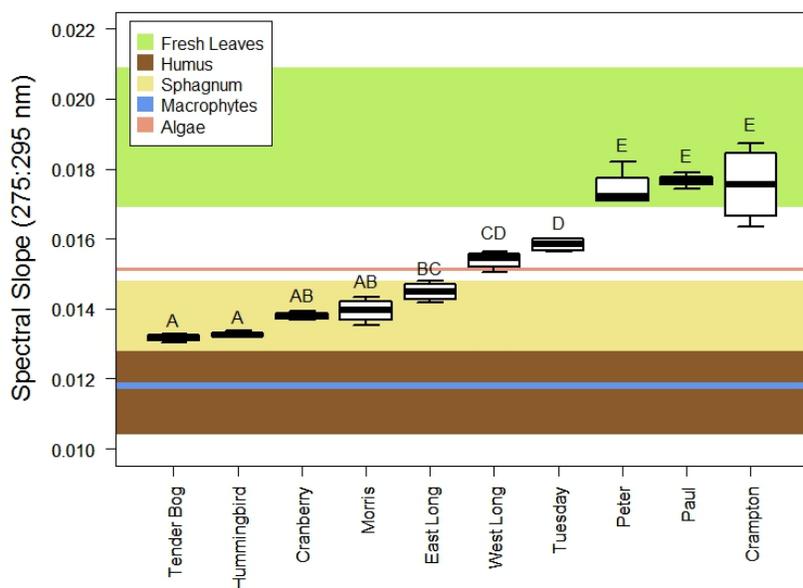


Figure 6. The distribution of $S_{275-295}$ across study lakes, in comparison with the leachate source spectral slopes (represented as the mean \pm standard deviation).

Table 3. Results of the post-hoc test (Tukey's Honest Significant Difference) for the difference of $S_{275-295}$ between lakes. *. The p-value is significant at the 0.5 level.

Lake (A)	Lake (B)	p-value
Cranberry	Crampton	<0.001*
East Long	Crampton	<0.001*
Hummingbird	Crampton	<0.001*
Morris	Crampton	<0.001*
Paul	Crampton	1.0000
Peter	Crampton	1.0000
Tender Bog	Crampton	<0.001*
Tuesday	Crampton	<0.001*
West Long	Crampton	<0.001*
East Long	Cranberry	0.4277
Hummingbird	Cranberry	0.7613
Morris	Cranberry	0.9999
Paul	Cranberry	<0.001*
Peter	Cranberry	<0.001*
Tender Bog	Cranberry	0.5681
Tuesday	Cranberry	<0.001*
West Long	Cranberry	<0.001*
Hummingbird	East Long	0.0111*
Morris	East Long	0.7495
Paul	East Long	<0.001*
Peter	East Long	<0.001*
Tender Bog	East Long	0.0050*
Tuesday	East Long	0.1308
West Long	East Long	0.0038*
Morris	Hummingbird	0.4401
Paul	Hummingbird	<0.001*
Peter	Hummingbird	<0.001*
Tender Bog	Hummingbird	1.0000
Tuesday	Hummingbird	<0.001*
West Long	Hummingbird	<0.001*
Paul	Morris	<0.001*
Peter	Morris	<0.001*
Tender Bog	Morris	0.2725
Tuesday	Morris	0.0017*
West Long	Morris	<0.001*
Peter	Paul	0.9985
Tender Bog	Paul	<0.001*
Tuesday	Paul	<0.001*
West Long	Paul	<0.001*

Tender Bog	Peter	<0.001*
Tuesday	Peter	<0.001*
West Long	Peter	<0.001*
Tuesday	Tender Bog	<0.001*
West Long	Tender Bog	<0.001*
West Long	Tuesday	0.8933
