Characterization of bulk and chromophoric dissolved organic matter in the Laurentian Great Lakes during summer 2013

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Abstract

The Laurentian Great Lakes contain ~21% of earth's surface freshwater and are experiencing dramatic decadal-scale changes to their water quality, biogeochemistry and ecosystem functions. We report here the first data set of dissolved organic carbon (DOC) and chromophoric dissolved organic matter (DOM), including UV-visible absorbance and derived optical properties and fluorescence excitation-emission matrices (EEMs), for open-lake surface-waters from each of the Great Lakes. Concentrations of DOC ranged from 86 to 240 µmol/L and the absorption coefficient at 254-nm (a_{254}) varied from 2.67 to 10.47 m^{-1} in Great Lakes waters. UV-visible and fluorescence characterization indicated that the DOM in open-lake waters in all the Great Lakes was primarily autochthonous. Both DOC and a_{254} increased from Lake Superior to Lakes Erie and Ontario where aromaticity, molecular-weight and humification-degree of DOM were also higher. These variations are consistent with both the water transport pathway and the trend of increasing human population and relative watershed drainage area along the Great Lakes. Parallel factor analysis on fluorescence-EEM data revealed three terrestrial humic-like DOM components (C1, C2 and C4) and one protein-like DOM component (C3). Open Lake Huron had the highest protein-like/humic-like DOM ratio (C3/C1), while sites in Lakes Erie and Ontario had lower C3/C1 ratios, indicating a greater proportional influence of terrestrial DOM. Changes in DOM composition along the water transport pathway in the Great Lakes indicate varying impacts from terrestrial inputs and human activities in the different lake systems.

Keywords: Dissolved organic matter, CDOM, fluorescence EEMs, PARAFAC analysis, the Laurentian Great Lakes.

Introduction

Lake ecosystems play an important role in water and carbon cycles (Cole et al. 2007, Tranvik et al. 2009) and serve as sentinels, integrators, and regulators of climate and environmental change (Moss 2012, Williamson et al. 2009). Within lakes themselves, dissolved organic matter (DOM), the largest water-column organic carbon pool, acts as a regulator of environmental quality and ecosystem functions. The presence of DOM can reduce the availability of ultraviolet radiation (UVR), change the thermal structure of the water column, affect microbial productivity, modify aquatic food structure, and mediate impacts of anthropogenic stressors (Prairie 2008, Williamson et al. 2009).

Chromophoric DOM (CDOM) has been measured in a variety of lakes, providing a concentration proxy, degradation or diagenetic status, and inferred structural characterizations of bulk DOM (Cuthbert and del Giorgio 1992, McKnight et al. 1997, Morris and Hargreaves 1997; Wu et al. 2003, Hua et al. 2010, Yao et al. 2011). More recently, fluorescence excitation-emission matrix (EEM) techniques coupled with parallel factor (PARAFAC) analysis have been widely used to investigate characteristics of DOM in aquatic systems, including lake waters (Mostofa et al. 2005, Wang et al. 2007, Hiriart-Baer et al. 2008, Fellman et al. 2010, Osburn et al. 2011, Yuan et al. 2014, Zhao et al. 2016). However, reports on characterization of CDOM and fluorescent-DOM (FDOM) in the Laurentian Great Lakes remain scarce, despite their usefulness in evaluating environmental quality and providing insights into DOM sources and reactions in aquatic systems. Studies reporting bulk dissolved organic carbon (DOC), CDOM, and FDOM coupled with PARAFAC for all the Great Lakes are even fewer.

The Laurentian Great Lakes, consisting of Lakes Superior (LS), Huron (LH), Michigan (LM), Saint Clair (LSC), Erie (LE), and Ontario (LO), are the largest connected group of freshwater

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lakes by surface area on Earth (Meyers 2006), holding nearly 21% of global surface freshwaters. Over the last few decades, the Great Lakes have experienced significant ecological and environmental changes due to the introduction of invasive species, such as zebra mussel and quagga mussel, and increasing anthropogenic influences, resulting in changes in ecosystem function and food web structure (Cuhel and Aguilar 2013, Vanderploeg et al. 2010; Bunnell et al., 2014) and increasing hypoxia extent (Scavia et al. 2014, Zhou et al. 2015). Nevertheless, the impacts of these ecological and environmental changes on water quality and carbon dynamics remain poorly understood, in part because of the lack of inter-lake historical data sets. Nutrient and carbon concentrations and composition have been investigated in the Great Lakes to examine controlling factors in nitrogen, phosphorus, and carbon biogeochemical cycling (Finlay et al. 2013, Hecky et al. 2004, Kane et al. 2014); however, most Great Lakes studies have generally been focused on individual lakes (e.g., Cotner et al. 2004, Minor and Stephens 2008, Zigah et al. 2011, Lin and Guo, 2016). Light absorbing properties have been determined across the Great Lakes in order to provide key data for remote sensing applications (Perkins et al. 2013). They identified the largest absorption contribution at 440 nm as coming from CDOM, and provided general CDOM spatial distribution patterns. In addition, fluorescence EEMs spectroscopy combined with PARAFAC analysis has been used for identification of DOM components in coastal waters of Lakes Erie and Ontario (Hiriart-Baer et al. 2008), but, again, as for carbon data, not for all the Laurentian Great Lakes.

In the present study, water samples were collected from each of the Laurentian Great Lakes during summer 2013, for the measurements of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), UV-vis absorbance, and fluorescence EEMs in addition to hydrographic data and other derived optical properties such as specific UV absorbance (SUVA) and spectral

slopes. PARAFAC analysis was then applied to EEMs data to identify major fluorescent DOM components. The primary objective was to provide a summer baseline dataset on the characteristics of DOM, including bulk DOC, CDOM and FDOM, in Great Lakes ecosystems and to identify trends in abundance, composition, sources and transformation processes along the water transport pathway from the upper Great Lakes (Lake Superior) to the lower Great Lakes (Lakes Erie and Ontario).

Methods

Sample collection

   Samples from Lake Michigan, Lake Huron, Lake St. Clair, Lake Erie and Lake Ontario were collected on board the R/V *Sea Dragon* in cooperation with the Pangaea exploration (http://panexplore.com) between August 19 and September 24, 2013 (Table 1, Fig. 1). Water temperature and specific conductivity were determined using a handheld YSI meter (YSI Incorporated). Samples were immediately filtered with pre-cleaned 0.7 µm glass fiber syringe filters (Whatman). The first 10 ml of filtrate was discarded. Filtrates were kept in a refrigerator and shipped to the lab in iced coolers. Water samples from Lake Superior were collected on September 16, 2013 from the R/V *Blue Heron* using a rosette and ancillary data were collected using a SBE9 CTD (Sea-Bird Scientific). These samples were shipped to the laboratory at Milwaukee in an iced cooler and then filtered through pre-combusted 0.7 µm GF/F filters (Whatman). Selected samples with large volumes were also filtered through both GF/F filters and syringe filters. No significant difference was found in their CDOM absorbance.

   To provide further information on Lake Superior's DOM and inorganic carbon pools, data from samples collected in summer 2010 are also included in the results and discussion below. These samples were collected from the R/V *Blue Heron* via CTD rosette. Inorganic carbon

samples were preserved and organic carbon samples were filtered (and acidified for DOC analysis) on shipboard as described in Zigah et al., 2014 (see Table 1 for further details).

Measurements of DOC and DIC

For samples collected in 2013, concentrations of DOC were measured with a Shimadzu TOC-L analyzer (Shimadzu Corp.) using the high temperature combustion method (Guo et al. 1995). Calibration curves were generated each analytical day before sample analysis. Three to five replicate measurements, each injecting 150 µL sample, were performed for each sample. The detection limit was 0.6 µmol-C/L and the precision and accuracy were better than 2% in terms of coefficient of variation. Ultrapure water, working standards, and consensus reference DOC samples from University of Miami were measured every eight samples to ensure data quality (Zhou and Guo 2012). Concentrations of total dissolved carbon (TDC) were measured in un-acidified samples. The concentration of DIC was calculated from the difference between TDC and DOC concentrations, DIC = TDC – DOC (Guo and Macdonald 2006). All samples were measured within a week of sample arrival to the lab at Milwaukee. DOC and DIC of the samples from an additional four Lake Superior stations were obtained using a Shimadzu V_{CSH} analyzer at the Large Lakes Observatory (Duluth, MN) following protocols in (Zigah et al. 2011).

Measurements of UV-vis absorption

For the 2013 samples, the UV-vis absorption spectra were measured on a Agilent 8453 spectrophotometer (Agilent) using a 10-mm quartz cuvette over 200-1100 nm with 1 nm increments (Zhou et al. 2013). Prior to analysis, the samples were allowed to warm up to room temperature. The water/filter blank was subtracted, and the refractive index effect was corrected by subtracting the average absorbance between 650 and 800 nm (Stedmon and Bro 2008). Both

reabsorption and inner filter effects were minimal for all the samples by sample dilution if needed (Ohno 2002, Guéguen et al. 2012; Zhou et al., 2013).

For the 2010 Lake Superior samples, spectra were determined using a Genesys 6 scanning spectrophotometer (ThermoFisher Scientific) and a 50 mm quartz cuvette over 200 to 800 nm in 1 nm increments. Water blank was subtracted.

Absorption coefficients were calculated from the equation: $a_{(\lambda)} = A_{(\lambda)} \times 2.303/l$, where $a_{(\lambda)}$ is the absorption coefficient at a specific wavelength (λ), $A_{(\lambda)}$ is the absorbance at wavelength λ, and l is the light path length in meters. Values of specific UV absorbance ($SUVA_{254}$) were calculated by normalizing the UV absorbance at 254 nm (m^{-1}) to the DOC concentration (mg C L^{-1}), as a measure of DOM aromaticity (Aiken et al. 2011, Spencer et al. 2010, Weishaar et al. 2003). Spectral slope ($S_{275-295}$) was calculated using linear regression of absorption coefficients over the wavelength interval of 275-295 nm and slope ratio (S_R) was calculated from ratio between the linearly fitted $S_{275-295}$ and $S_{350-400}$ (Helms et al. 2008).

Non-chromophoric DOC

The abundance of non-chromophoric DOC was defined as the DOC concentration at zero aromaticity or $a_{254} = 0$ (e.g., Spencer et al. 2008). Thus, it can be derived from the intercept value in the plot of DOC against absorption coefficient and represents the DOC fraction that does not absorb light (DeVilbiss et al. 2016).

Measurements of fluorescence EEMs and applications of PARAFAC analysis

Fluorescence excitation–emission matrix (EEM) spectra were measured on a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon) running the software FluoEssence V3.5 and using a 10 mm UV-grade quartz cuvette. Each fluorescence EEM was concatenated from emission spectra

recorded over the range of 240-600 nm with 1 nm steps under excitation from 220 to 480 nm with 2 nm steps (Zhou et al. 2013). Scans of ultrapure water were subtracted from the sample spectra before EEM contour plots were generated. A series of quinine sulfate solutions in 0.05 M H_2SO_4 were measured at Ex/Em 350/450 nm. The fluorescence intensity of 1 ppb quinine sulfate obtained through regression was used for normalization of the blank-corrected sample spectra to quinine sulfate equivalents (ppb QSE) (Coble et al. 1998). The areas in the EEMs that were interfered with by the first and second order Raman and Rayleigh scattering peaks were eliminated from the dataset (Murphy et al. 2013). Instrument response correction was done by manufacturer-generated correction factors (Cory et al. 2010). All PARAFAC analyses were performed in MATLAB 6.5.1 (Mathworks) using the DOMfluor toolbox (Stedmon and Bro 2008). Additional EEM data from Lake Michigan samples were combined for the PARAFAC analysis (a total of 135 samples, including 21 from the 2013 cross-lakes sampling and 114 from Lake Michigan). The model was constrained to nonnegative values and the results were validated using split half analysis (Andersen and Bro 2003, Stedmon et al. 2003).

The biological index (BIX) was calculated as the ratio of fluorescence intensity at 380 nm to that at 430 nm under the excitation wavelength of 310 nm (Huguet et al. 2009). The humification index (HIX) was obtained from the ratio of the integration of fluorescence between the 300-345 nm and 435-480 nm over excitation at 254 nm (Huguet et al. 2009, Zsolnay et al. 1999).

Data statistics

  All statistical analyses were done in MATLAB 6.5.1 (Mathworks). One-way ANOVA tests were performed to examine significance levels of correlations.

Results and Discussion

Physicochemical characteristics

Water temperature, specific conductivity (SPC), and DIC concentrations are listed in Table 1. Surface water temperature varied latitudinally, and as a function of lake water depth, with the lowest values found in the northernmost and deepest lake (Lake Superior) and the highest value found at the southern most sampling site in shallow Lake Erie (Table 1). Based on satellite imaging of Chl-*a* (Fig. 2a, http://oceancolor.gsfc.nasa.gov/) and upon analysis of filtered water samples (Fig. 2b, data from http://www.epa.gov/greatlakes/monitoring/data_proj/glenda) in the Great Lakes during August 2013, Lakes Superior, Michigan and Huron had lower Chl-*a* concentrations, suggesting relatively more oligotrophic conditions compared to Lakes Erie and Ontario, where higher Chl-*a* values were found, especially in the west of these two lakes. Higher Chl-*a* values in Lakes Erie and Ontario were likely related to more eutrophic conditions, in part from higher nutrient inputs from the more agricultural and warmer watersheds in the lower Great Lakes (Kane et al. 2014). Relatively higher Chl-*a* concentrations were also found in northern Lake Michigan, western Lake Huron and northern Lake Superior, especially nearshore (Fig. 2a), although in such regions the satellite data may be impacted by the presence of sediment and CDOM. It is difficult to tease apart the optical influences of chlorophyll, sediment, and CDOM in coastal waters. Compared to the other Great Lakes, Lakes Erie and Ontario are relatively small in both surface area and volume, receive accumulated influences from upstream Great Lakes, and have considerable local inputs from rivers, streams, and groundwater (Quinn 1992, Scavia et al. 2014, Zhou et al. 2015). This can lead to higher inputs of terrestrially-derived organic matter and also nutrients, which can support higher Chl-*a* concentrations in the lower two lakes at a whole-lake level.

As shown in Table 1, lowest SPC (~100 µS cm^{-1}) was found in Lake Superior waters, while

highest SPC (279 µS cm^{-1}) was found in Lake Michigan. Concentrations of DIC varied from 775

to 2107 µmol L^{-1} (Table 1), with the highest values found in Lake Michigan as well, exhibiting a

similar spatial distribution pattern as SPC. Compared to other Great Lakes, Lake Michigan

possessed significantly higher SPC, DIC and alkalinity (not shown), consistent with the wide

distribution of limestone in the drainage basin in Lake Michigan (Binford and Quimby 1963).

Variations in DOC and UV-absorbance

   Concentrations of DOC in open lake surface waters in the Great Lakes varied from 86 to 240

µmol L^{-1}, with an average of 156±49 µmol L^{-1} (Table 2 and Fig. 3). In general, DOC

concentrations were the highest in Lake Erie (ranging from 200-240 µmol L^{-1}, with an average of

214±18 µmol L^{-1}) followed by Lake Ontario (ranging from 184-204 µmol L^{-1}, with an average of

195±8 µmol L^{-1}), and the lowest DOC concentration was measured in open Lake Superior

(ranging from 86-101 µmol L^{-1}, with an average of 92±6 µmol L^{-1}, Fig. 4). These DOC

concentrations in the open Great Lakes are considerably lower than those reported for smaller

lakes in eastern Canada (283-688 µmol L^{-1} (Zhang et al. 2010)). Open Lake Michigan waters

had an average DOC concentration of 183±22 µmol L^{-1}, which is almost the same as those

reported for southern Lake Michigan during 2000-2001 (Biddanda and Cotner 2002), although

DOC concentrations in Lake Michigan seemed to increase from the south to the north of the lake

(Table 2).

   Concentrations of DOC (Fig. 3) had distribution patterns similar to those of Chl-*a* (Fig. 2a),

and were also linearly correlated (r^2 = 0.74, p = 0.06) with the ratio of watershed drainage area to

lake surface area (RWS) and thus with terrestrial impact on lake waters (Fig. 4). For example,

Lake Erie has a relatively higher (~3.0) RWS (Cotner et al. 2004) and the watershed of Lake Erie

supports about a third of the population residing in the Great Lakes region, making it more exposed to urbanization, agricultural activities, and higher terrestrial DOM fluxes as well as the high primary productivity associated with eutrophication (Bridgeman et al. 2012). Consistent with these trends, western Lake Erie had the highest DOC concentration of all samples (Table 2). The highest DOC concentration (221 µmol L^{-1}) in Lake Michigan was found at station LM-05 in the north of the lake, reflecting influences from the Fox River and Green Bay (Lin et al., 2016), where DOC concentrations were 334±78 µmol L^{-1} (DeVilbiss et al. 2016). Lake Superior had the lowest RWS (1.55) among all the Great Lakes (Cotner et al. 2004), the lowest human population within its watershed, and little ecosystem perturbation (Dobiesz et al. 2010), leading to the lowest open-lake DOC concentrations. Comparisons of the average DOC concentration in each lake exhibited a general increasing trend of DOC from the northwest to the southeast in the Great Lakes (Fig. 4), following the main water flow pathway and the trend of increasing watershed population density from Lake Huron to Lake Erie and Lake Ontario (Dobiesz et al. 2010), with the lowest DOC abundance in Lake Superior, a slight increase in Lake Michigan and Lake Huron, and the highest in Lakes Erie and Ontario (Table 2 and Fig. 4).

The absorption coefficient at 254 nm (a_{254}) ranged from 3.57 to 10.47 m^{-1} (Table 2), and had distributions similar to those of DOC (Fig. 5). SUVA$_{254}$ values ranged from 0.97 to 1.58 m^2 (gC^{-1}) (Table 2), with the lowest value found in the middle of Lake Huron. The highest SUVA$_{254}$ values were found at stations in western Lake Erie and northern Lake Michigan, the same stations where highest DOC and a_{254} values were found (Fig. 3), suggesting that a larger proportion of that DOC is highly aromatic and derived from terrestrial sources. Within Lake Superior, the highest SUVA$_{254}$ value was found at the westernmost, most nearshore station and decreased farther offshore (Fig. 3). Spectral slope ($S_{275-295}$) ranged from 0.0242 to 0.0328 nm^{-1} in

the Great Lakes, with the lowest value found in western Lake Erie, where the highest DOC, a_{254}

and SUVA$_{254}$ were measured, and the highest S$_{275-295}$ value found in the middle of Lake Huron,

matching the lowest SUVA$_{254}$. Relatively lower S$_{275-295}$ values (i.e. higher DOM molecular

weight) were also found in western Lake Superior near shore and in northern Lake Michigan.

The averaged slope ratio (S$_R$) clearly showed higher values in Lake Superior and lower values in

Lake Erie (Fig. 4), consistent with the generally lower DOC and CDOM abundance in Lake

Superior. A linear negative correlation ($r^2 = 0.82$, $p < 0.09$) was found between the RWS and the

average S$_R$ in each lake (Fig. 4). Lakes with lower RWS and longer water residence times likely

receive less input of larger-sized terrestrial DOM and produce more reworked DOM through

processes that could also convert terrigenous organic matter into recalcitrant low-molecular-

weight compounds (Urban et al. 2005).

A significant positive correlation was observed between DOC and a_{254} (r^2=0.86, p<0.0001,

Fig. 5). However, as discussed above, DOC in open Lake Superior was particularly low

compared to other lakes, likely resulting from limited terrestrial inputs, its oligotrophic setting,

and extensive reprocessing in the water column due to both the longer water residence time and

water clarity (Binding et al. 2015), which allow extensive photochemical processing. There was

a distinct relationship between DOC and a_{254} in Lake Superior as compared to the rest of Great

Lakes (Fig. 5). Indeed, upon exclusion of data points from Lake Superior, a better correlation

between DOC and a_{254} was found ($r^2 = 0.92$, p <0.00001) in the pooled data from the other Great

Lakes. Differences in DOM characteristics between Lake Superior and other Great Lakes are

also manifested in the abundance of non-chromophoric DOC defined as the DOC concentration

at zero aromaticity or $a_{254} = 0$ (Spencer et al. 2008). For example, the non-chromophoric DOC

was ~56 µmol L^{-1} in Lake Superior and 92 µmol L^{-1} for the combination of all the other

Laurentian Great lakes (Fig. 5), representing 61% and 50% of the bulk DOC in Lake Superior and the combined other lakes, respectively. A higher percentage of non-chromophoric DOC in Lake Superior indicates limited terrestrial DOM and/or more intense photo-degradation in Lake Superior compared to the rest of the Great Lakes (Biddanda and Cotner 2003).

Table 3 lists the abundance of non-chromophoric DOC and its percentage in the bulk DOC from different freshwater environments. In general, the open-water Great Lakes show a lower concentration in non-chromophoric DOC, but a larger proportion of non-chromophoric DOC in the bulk DOC, than river systems and smaller embayments (Fig. 5). Interestingly, the non-chromophoric DOC abundance seems to increase with increasing bulk DOC concentration, but the percentage of non-chromophoric DOC shows a significant negative correlation with bulk DOC concentration based on available data sets ($r^2 = 0.93$, $p = 0.0001$, Fig. 5). This seems to suggest production of non-chromophoric DOM from the aromatic DOM combined with the effects of shielding on photo-degradation of DOM in the water column.

Concentrations of DOC were significantly inversely correlated with spectral slope ratio, S_R, in the Great Lakes ($r^2 = 0.45$, p <0.002, Fig. 5), thus indicating higher molecular weight DOM when DOC concentrations are high. Combining data trends, higher DOC samples with higher aromaticity generally contain more HMW-DOM in Great Lakes waters.

Fluorescent DOM characteristics

Major characteristics of fluorescent DOM can be obtained from EEM spectra (Coble 1996) although more specific fluorescent DOM components can be derived from coupling EEM data with PARAFAC analysis (see section 3.4). As shown in Fig. 6, fluorescence EEM spectra in the Great Lakes were characterized by major signatures of humic-like (Peak A) and protein-like (Peak B and T) DOM (Coble 1996), showing both allochthonous and autochthonous sources

(Coble 1996, Stolpe et al. 2014). In addition, minor signatures of peaks C and M were also present (Fig. 6). Peak C has been shown to be linked to terrestrial humic-like DOM, while peak M was traditionally recognized as autochthonous DOM (Coble 1996). Recently, peak M has also been attributed to microbially altered DOM (Guéguen et al. 2011, Stedmon and Markager 2005b; Hur et al., 2009).

There seems to be a shift in the peak position for protein-like fluorophores in the Great Lakes, which can be quantified as a change in the Ex/Em wavelengths at maximum intensity as listed in Table 4. From Lake Superior to Lake Ontario, the peak positions generally showed a trend of shifting from a tyrosine-like (peak B, Ex/Em 280/320 nm) to a tryptophan-like DOM (peak T, at Ex/Em 285/342 nm) from west to east in the Great Lakes. As discussed above, Chl-a concentration and bulk DOM molecular weight as inferred from S_R both exhibited an increasing trend from Lake Superior to Lake Ontario, suggesting increasing freshly produced protein-like DOM with higher molecular weight from west to east in the Great Lakes. Indeed, peak B has been found to be subject to quenching due to energy transfer when tyrosine is bound to larger structures of HMW proteins (Lakowicz 1999, Maie et al. 2007, Yamashita and Tanoue 2004). Thus, the shift of protein-like fluorophores from peak B in Lake Superior to peak T in Lake Ontario suggests increased *in situ* production and bacterial activities associated with eutrophication and elevated abundance of fresh, large-structure proteins (e.g., Romera-Castillo et al. 2011). Similar shift from protein-like to humic-like fluorophores with eutrophication was observed in other lake environments (e.g., Zhang et al. 2010).

Indices derived from EEM data could also provide complementary information for DOM characterization. For example, the humification index (HIX) has been used as an indicator of the age and recalcitrance of DOM, with higher HIX generally representing increased degree of

decomposition or processing (Huguet et al. 2009). In Great Lakes open waters, values of HIX varied from 0.9 to 2.9 (Table 4), with the lowest in Lake Huron (Table 4, Fig. 7) and the highest values found in both Lakes Erie and Ontario. Notice that, all HIX values were well below 5 for the open lake water samples. HIX< 5 has been found to correlate with biological production and a lower degree of decomposition and to characterize offshore waters (Huguet et al. 2009, Hunt and Ohno 2007, Wickland et al. 2007). Our HIX values therefore suggest that DOM in the open Great Lakes contains higher proportions of freshly produced organic matter, with relatively less terrestrial influence compared to estuarine, nearshore (flood-impacted) marine, and forest stream environments (Bianchi et al. 2013, Huguet et al. 2009, Yang et al. 2015).

The biological index (BIX), in contrast, is indicative of autochthonous DOM in natural waters, with higher BIX values linked to more *in situ* production (Huguet et al. 2009). In the Great Lakes, BIX ranged from 0.84 to 0.97 (Table 4), with low values observed in the northern Lake Michigan, Lake Superior, and other stations where higher HIX was found. The highest BIX value was found in middle Lake Huron (Fig. 7). Generally, higher BIX values were found at the lower latitudes (likely higher temperature) open lake stations, where increased DOM could originate from autochthonous sources. Previous studies have reported that BIX values between 0.8 and 1.0 indicate a large proportion of freshly produced DOM from biological/microbial origin, while DOM with BIX values lower than 0.6 indicate little autochthonous DOM (Huguet et al. 2009). As shown in Table 4, BIX values in open Great Lakes surface waters were relatively high, indicating that DOM in the open waters of the Great Lakes contains a strong autochthonous signature, consistent with increasing water clarity in the Great Lakes (e.g., Binding et al. 2015) and thus enhanced photodegradation of terrestrial DOM.

Major fluorescent DOM components derived from PARAFAC analysis

Four major DOM components were identified from the Great Lakes based on PARAFAC modeling of the EEMs data (n=135 samples). Three of the DOM components (C1, C2 and C4) are humic-like materials and one component (C3) showed characteristics of protein-like DOM (Table 5 and Fig. 8). Component 1 (C1, Ex/Em of 255/464 nm and an additional minor peak at Ex/Em ~340/460 nm) resembles the traditional peaks A and C in previous studies (Coble 1996), showing characteristics of humic-like DOM. C2 (Ex/Em of 245/394 and ~315/390 nm, respectively) can be categorized as microbial-altered terrestrial humic materials (Kowalczuk et al. 2009, Stedmon et al. 2003). C3 (Ex/Em of 280/324 nm, with the emission spectra of the peak extending to ~340 nm) resembles peak T (Coble 1996), a protein-like component (Murphy et al. 2008, Stedmon and Markager 2005a, b). C4 (Ex/Em of 275/508 nm) is a terrestrial organic material with higher MW and aromaticity (McKnight et al. 2001, Stedmon et al. 2003).

Distributions of DOM components (C1, C2 and C4) showed their highest abundance in western Lake Erie, northern Lake Ontario and northern Lake Michigan (Fig. 9), where higher HIX values were found (Fig. 7). The strong presence of C1, C2 and C4 in both Lakes Erie and Ontario (Table 4) is similar to the spatial distribution of bulk DOC concentration. The distribution of C3, the protein-like DOM component, exhibited some association with Chl-*a* (Figs. 2 and 9), showing higher values in Lakes Erie and Ontario, northern Lake Michigan (LM-05) and western Lake Huron (LH-01 and 02), indicating sources of protein-like DOM from *in situ* production in these relatively more eutrophic areas.

In general, the PARAFAC-derived DOM components revealed distinct characteristics of humic-like and protein-like DOM and region-specific distribution patterns (Fig. 9). The proportions (or relative abundances) of humic-like and protein-like DOM within the bulk DOC could be represented by the ratios of C1/DOC and C3/DOC, respectively. As shown in Fig. 10,

the C1/DOC and C3/DOC ratios both exhibited higher values in western Lake Erie (0.052 and

0.033 gQSE $(molC)^{-1}$, respectively), northern Lake Ontario (0.063 and 0.039 g-QS/mol-C) and

northern Lake Michigan (0.026 and 0.028 g QSE $(mol\ C)^{-1}$), reflecting larger proportions of both

humic-like and protein-like materials in the bulk DOM in these areas (Fig. 10). The ratio

between a protein-like component and a terrestrial humic-like component has proven useful in

tracking *in situ* production (Guéguen et al. 2011). Here, the C3/C1 ratio was used as a proxy for

the relative importance of autochthonous vs allochthonous DOM, as further supported by the

significant negative correlation between the C3/C1 ratio and HIX (Table 4, $r^2 = 0.81$, *p*<0.00001).

A decrease in the importance of humified DOM was accompanied by more pronounced *in situ*

production of protein-like DOM. As shown in Fig. 10, the highest C3/C1 ratio (2.2) was found in

mid-Lake Huron, where terrestrial sources are likely at a minimum level and chlorophyll

concentrations were moderately high. Similarly, high C3/C1 ratios (1.6-1.9) were also found in

open Lake Michigan. On the other hand, the lowest C3/C1 ratios were found in western Lake

Erie (0.6) and northern Lake Ontario (0.6), even though the relative concentrations of both C1

and C3 in the bulk DOC were higher. It seems that these regions received significant terrestrial

inputs, and *in situ* biological activities did not contribute as high a proportion to the total DOM

pool as in other sampling locations. Hypoxia has been a regular summertime occurrence in Lake

Erie (Kane et al. 2014, Scavia et al. 2014). Our results indicate that higher allochthonous inputs

likely play an important role in the development of eutrophication and hypoxia in these areas in

addition to nutrient enrichment (and subsequent primary production) and physical/hydrological

conditions.

Conclusions

Lakes Erie and Ontario and northern Lake Michigan were characterized by higher DOC, a_{254}, SUVA$_{254}$ and HIX values, and lower $S_{275-295}$, S_R and BIX values, or higher aromaticity, higher molecular weight, and extensive humification. In contrast, DOM in central Lakes Huron and Michigan was characterized by lower SUVA$_{254}$ and HIX values, but higher $S_{275-295}$ and BIX, reflecting a change in DOM sources and cycling processes along the water transport pathway from the upper to lower Great Lakes. Lake Superior had the lowest non-chromophoric DOC abundance but the highest percentage of this DOC pool within bulk DOC, most likely resulting from limited terrestrial DOM inputs and more influence from photo-degradation. Four fluorescent-DOM components were identified based on PARAFAC analysis: three of them (C1, C2 and C4) were humic-like DOM and one (C3) was protein-like DOM. Higher fluorescent-DOM abundances but lower C3/C1 ratios were observed in Lakes Erie and Ontario and northern Lake Michigan, where higher terrestrial inputs and hypoxia have been reported. Higher C3/C1 ratios were observed at open stations in Lakes Huron and Michigan, showing the importance of autochthonous DOM in the open lake. Based upon our summer sampling across the Great Lakes, inputs of terrestrial organic matter and in-lake organic matter production from associated nutrients seem to have an accumulative effect along the water transport pathway from Lake Superior to Lakes Erie and Ontario, and may play an important role in facilitating the development of hypoxia, especially in the lower Great Lakes. These geographic trends in biogeochemistry may also reflect the human population pressures on the individual watersheds in the lakes, which increase in roughly the same order. Further studies are needed to establish seasonal and annual baseline in carbon biogeochemistry in the Great Lakes and to examine impacts of climate and environmental changes in the Great Lakes ecosystem, such as the introduction of invasive species and the development of eutrophication and hypoxia.

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Table 1. Sampling locations, sampling dates, and hydrographic data for surface waters in the Great Lakes, including Lakes Superior (LS), Michigan (LM), Huron (LH), St. Clair (LSC), Erie (LE), and Ontario (LO).

Station	Latitude (^{o}N)	Longitude (^{o}W)	Sampling Date	Water depth (m)	Sampling depth (m)	Water Temp (°C)	Sp. Cond (μS cm^{-1})	DIC (μmol L^{-1})
LS-01	47˚18.96'	89˚50.82'	09/16/13	169.0	5	13.3	100.2	834
LM-01	41˚53.93'	87˚09.74'	08/19/13	45.0	0	22.6	251.0	2107
LM-02	42˚51.54'	87˚08.06'	08/19/13	78.6	0	21.2	253.0	2054
LM-03	43˚35.80'	87˚07.71'	08/20/13	110.0	0	20.3	230.0	2066
LM-04	44˚22.45'	87˚00.51'	08/20/13	210.0	0	19.9	279.0	2053
LM-05	45˚13.61'	86˚30.39'	08/21/13	159.0	0	18.9	278.0	2046
LH-01	45˚44.51'	83˚42.43'	08/24/13	116.4	0	17.7	187.0	1605
LH-02	45˚20.27'	83˚09.31'	08/24/13	116.1	0	19.0	175.0	1464
LH-03	44˚36.90'	81˚57.37'	08/25/13	164.6	0	19.4	204.0	1467
LH-04	43˚36.61'	82˚13.28'	08/25/13	48.5	0	21.1	187.0	1540
LSC-01	42˚27.83	82˚45.27'	08/26/13	7.8	0	22.9	195.0	1554
LE-01	41˚43.51'	82˚36.32'	09/03/13	10.1	0	23.5	268.0	1771
LE-02	41˚52.99'	81˚42.52'	09/06/13	24.1	0	23.4	259.0	1767
LE-03	42˚10.11'	80˚55.36'	09/06/13	23.2	0	22.6	247.0	1784
LE-04	42˚35.52'	79˚30.13'	09/07/13	53.6	0	21.8	266.0	1797
LO-01	43˚20.99'	79˚19.59'	09/08/13	105.2	0	19.4	256.0	1694
LO-02	43˚42.27'	78˚37.48'	09/23/13	104.8	0	19.4	256.0	1721
LO-03	43˚40.11'	77˚42.20'	09/24/13	135.1	0	19.4	256.0	1695
LO-04	43˚50.14'	76˚40.31'	09/24/13	50.3	0	19.4	256.0	1710
LS-BR	47˚20.06'	91˚11.42'	09/01/10	19	4	5.7	101.0	-
LS-WM	47˚19.24'	89˚51.52'	08/26/10	167	5	11.0	99.5	780[*]
LS-CM-01	48˚01.66'	87˚43.99'	08/31/10	253	5	17.1	100.3	-
LS-CM-02	48˚2.48'	87˚44.27'	08/31/10	253	37	4.2	102.1	-
LS-CM-03	48˚01.79'	87˚44.08'	08/31/10	253	190	3.7	100.6	-
LS-EM	47˚33.80'	87˚37.81'	08/30/10	243	5	17.3	99.2	775[*]
LS01-150m	47˚18.96'	89˚50.82'	09/16/13	169.0	150	3.7	102.7	838

[*]Data from (Zigah et al. 2014)

Table 2. Concentrations of dissolved organic carbon (DOC), absorption coefficient at 254 nm (a_{254}), specific UV absorbance at 254 nm (SUVA$_{254}$), spectral slope between 275-295 nm ($S_{275-295}$), and slope ratio (S_R) in the Great Lakes.

Station	DOC (μmol L^{-1})	a_{254} (m^{-1})	SUVA$_{254}$ (m^2 (gC)$^{-1}$	S_R	$S_{275-295}$ (nm^{-1})
LS-01	99	3.57	1.26	2.56	0.0278
LM-01	177	5.19	1.06	1.74	0.0312
LM-02	163	4.40	0.98	1.92	0.0323
LM-03	173	5.27	1.10	1.63	0.0326
LM-04	180	5.31	1.07	2.26	0.0304
LM-05	221	8.35	1.37	1.43	0.0272
LH-01	158	4.83	1.10	1.99	0.0303
LH-02	164	5.39	1.19	1.30	0.0299
LH-03	144	3.84	0.97	2.30	0.0328
LH-04	158	4.76	1.09	1.64	0.0313
LSC-01	154	4.11	0.97	1.70	0.0269
LE-01	240	10.47	1.58	1.44	0.0242
LE-02	208	7.41	1.29	1.57	0.0260
LE-03	200	6.99	1.27	1.74	0.0288
LE-04	210	6.91	1.19	1.74	0.0286
LO-01	204	6.47	1.15	1.65	0.0302
LO-02	196	6.42	1.19	1.76	0.0307
LO-03	195	6.55	1.22	1.74	0.0300
LO-04	184	6.24	1.23	1.48	0.0294
LS-BR	88	3.53	1.41	1.77	0.0286
LS-WM	89	3.39	1.26	1.97	0.0299
LS-CM-01	96	3.21	1.17	1.80	0.0341
LS-CM-02	88	3.11	1.29	2.41	0.0310
LS-CM-03	86	3.02	1.27	2.11	0.0317
LS-EM	88	2.67	1.06	2.56	0.0352
LS-01-150m	101	3.68	1.28	2.94	0.0272

Table 3. Comparisons in the abundance of non-chromophoric (non-CDOM) DOC in different freshwater systems. Data are listed in an order of ascending bulk DOC concentration.

Lake/River	Bulk DOC (μmol L^{-1})	Non-CDOM DOC (μmol L^{-1})	Non-CDOM DOC (%)	Reference
Lake Superior (LS)	92 ± 6	56	61	This study
Other Great Lakes (LM-LO)	185 ±26	92	50	This study
Mississippi River (MissR)	302 ± 41	138	46	Zhou (2012)
Green Bay-June-2014 (GB(Jun))	349 ± 64	157	45	DeVilbiss et al. (2016)
Green Bay-Aug-2014 (GB(Aug))	361 ± 73	119	33	DeVilbiss et al. (2016)
Pearl River (PR)	557 ± 222	120	21	Zhou (2012)
Yukon River (YR)	751 ± 539	121	16	(Spencer et al. 2008)
Milwaukee River (MilR)	824±246	239	29	Teber (2016)

Table 4. Excitation-emission (Ex-Em) wavelengths of Peaks T and B, biological index (BIX), humification index (HIX), abundance of major fluorescent DOM components (in ppb quinine sulfate equivalents, QSE) derived from PARAFAC analysis in the Great Lakes.

Station	Protein (Peak T &B)		BIX	HIX	C1 (ppbQSE)	C2 (ppbQSE)	C3 (ppbQSE)	C4 (ppbQSE)
	Ex (nm)	Em (nm)						
LS-01	280	320	0.86	1.32	1.73	1.19	2.19	0.77
LM-01	280	322	0.90	1.38	2.58	1.67	3.35	1.16
LM-02	280	320	0.90	0.98	1.56	0.92	2.95	0.69
LM-03	280	322	0.92	1.16	1.93	1.20	3.15	0.85
LM-04	280	320	0.91	1.24	2.40	1.55	3.47	1.08
LM-05	280	320	0.84	1.65	5.95	4.32	6.21	2.64
LH-01	280	320	0.88	1.15	2.43	1.69	3.95	1.08
LH-02	280	324	0.84	1.55	2.91	1.79	3.45	1.29
LH-03	280	322	0.97	0.90	1.24	0.83	2.72	0.53
LH-04	280	320	0.90	1.32	2.08	1.56	2.86	0.86
LSC-01	285	342	0.85	1.48	2.35	1.50	2.91	1.07
LE-01	285	344	0.88	2.86	12.47	9.20	7.83	6.14
LE-02	290	344	0.91	1.60	5.64	4.44	6.99	2.71
LE-03	285	344	0.91	1.83	5.17	3.91	5.38	2.41
LE-04	285	342	0.94	1.63	4.92	3.78	5.81	2.36
LO-01	285	342	0.95	1.37	4.08	3.04	5.75	1.89
LO-02	280	320	0.93	1.66	4.26	3.14	5.06	1.96
LO-03	290	360	0.87	2.90	12.20	9.01	7.54	6.09
LO-04	285	342	0.94	1.71	4.09	3.21	4.71	1.88

Table 5. Specific excitation-emission wavelengths of major fluorescent DOM components derived from PARAFAC analysis and their characteristics.

Component	Excitation wavelength (nm)	Emission wavelength (nm)	Description
Component-1	255, ~340	464	Terrestrial humic-like DOM
Component-2	245, ~315	394	Terrestrial humic materials altered by microbial reprocessing
Component-3	280	324-340	Protein-like
Component-4	275	508	Terrestrial humic-like, high molecular weight

Figure captions:

Fig. 1. Sampling locations (circles) in all the Laurentian Great Lakes, including Lakes Superior (LS), Michigan (LM), Huron (LH), Saint Clair (LSC), Erie (LE), and Ontario (LO) during August/September 2013. Additional samples from Lake Superior (squares) were taken in summer 2010, one of these sampling stations overlaps with the site covered in 2013.

Fig. 2. Spatial distributions of chlorophyll-a (upper panel (a) shows Chl-a image from NASA and middle panel (b) uses Chl-a data from Glenda, http://oceancolor.gsfc.nasa.gov/) and dissolved inorganic carbon (DIC, bottom panel (c)) in Great Lakes surface waters.

Fig. 3. Spatial distributions of dissolved organic carbon (DOC), absorption coefficient at 254 nm (a_{254}), specific UV absorbance (SUVA$_{254}$) and spectral slope ($S_{275-295}$) in the Great Lakes.

Fig. 4. Comparisons in average DOC concentrations and slope ratio (S_R) values (left panels) as well as the relationship between RWS (ratio of watershed drainage area to lake surface area) and DOC or S_R values (right panels) in the Laurentian Great Lakes. Note that LM* denotes the average values in LM excluding the data point near the Green Bay outlet.

Fig. 5. Correlations between (a) dissolved organic carbon (DOC, in $\mu mol\ L^{-1}$) with absorption coefficient (a_{254}), (b) spectral slope ratio (S_R) in the Great Lakes, (c) non-chromophoric DOC and (d) percentage (%) of non-chromophoric DOC (d) in different freshwater systems (See Table 3 for data sources).

Fig. 6. Examples of fluorescence EEM spectra from Great Lakes water samples, showing presence of humic-like DOM (Peaks A and C), protein-like DOM (Peaks B and T) and also bio-altered DOM with terrestrial sources (Peak M)

Fig. 7. Humification index (HIX) and biological index (BIX) in the Great Lakes. Notice that higher HIX and BIX values were both observed in Lakes Erie and Ontario, with different HIX and BIX values at different stations.

Fig. 8. Four major fluorescent DOM components and their excitation-emission spectra identified by PARAFAC analysis. Specific Ex/Em wavelengths for the four DOM components are listed in Table 5.

Fig. 9. Distributions of the four fluorescent DOM components (in quinine sulfate equivalents-ppb, ppb-QSE) at different stations (a) and their percentages (b) in the Great Lakes.

Fig. 10. Spatial distributions of the relative abundance of DOM components, C1 and C3, in the bulk DOC (or C3/DOC and C1/DOC, respectively) and the protein-like/humic-like ratio (C3/C1) in the Great Lakes.

Fig. 1.

Fig. 2.

a

Chlorophyll a concentration (mg / m³)

0.01   0.03   0.1   0.3   1   3   10

b

Chla (mg m⁻³) in the Great Lakes

c

DIC (μmol L⁻¹) in the Great Lakes

Fig. 3.

DOC (μmol L^{-1}) in the Great Lakes

a_{254} (m^{-1}) in the Great Lakes

SUVA$_{254}$ (m^2 g–C^{-1}) in the Great Lakes

Spectral Slope (nm^{-1}) in the Great Lakes

Fig. 4.

Fig. 5.

Fig. 6

Lake Superior–05m–09242013

Lake Michigan–03–0m–08202013

Lake Huron–04–0m–08252013

Lake St Clair–01–0m–08262013

Lake Erie–03–0m–09102013
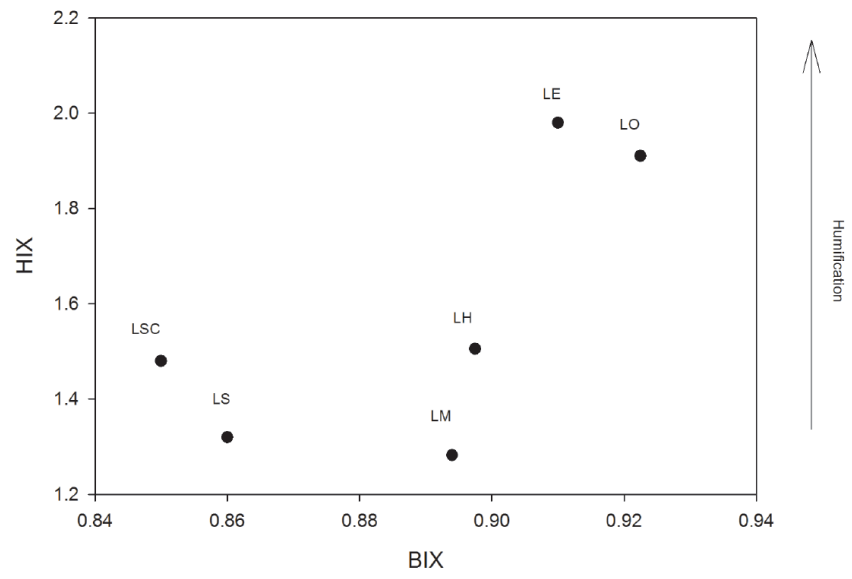
Lake Ontario–04–0m–09242013

Figure 7.

Fig. 8.

Component 1

Component 2

Component 3

Component 4

Figure 9.

Fig. 10.

C1/DOC (g–QS mol–C^{-1}) in the Great Lakes – Aug Sep 2013

C3/DOC (g–QS mol–C^{-1}) in the Great Lakes – Aug Sep 2013

C3/C1 in the Great Lakes – Aug Sep 2013