

## Article

# Dissolved organic matter quantity and quality in Lake Simcoe compared to two other large lakes in southern Ontario

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## Abstract

Dissolved organic matter (DOM) is a critical component in ecosystem processes and is the largest pool of organic carbon (C) in aquatic environments. In this study, we investigated the variability in quantity and quality of DOM in 3 large lakes in southern Ontario. Water quality parameters were coupled with excitation emission fluorescence spectroscopy and absorption spectra to characterize the DOM and investigate the overarching factors controlling DOM dynamics. The results show that Lake Simcoe has higher dissolved organic carbon (DOC) concentrations than lakes Erie, Ontario, and Hamilton Harbour (an embayment in western Lake Ontario) and suggest that a DOM source independent of watershed inputs is likely an important contributor to the DOC in this system. Five components were identified through parallel factor analysis (PARAFAC), representative of both terrestrial and microbial origin. Their relative intensities in the 4 Lake Simcoe end-members allowed the identification of dominant DOM sources in our studied ecosystems. Lake Simcoe seems to have a similar contribution of agriculturally derived DOM to lakes Erie, Ontario, and Hamilton Harbour. Lake Ontario, including Hamilton Harbour had on average a larger input of DOM derived from wastewater treatment plant effluents. The seasonal patterns in the different optical characteristics of DOM in Lake Simcoe compared to other systems suggested that DOM qualitative transformations, be it through photooxidation or microbial degradation, are likely very important processes in this lake. The role of DOM in Lake Simcoe may have important ecological implications for the cycling of C and the oxygen regime of this lake.

**Key words:** absorption, DOM, fluorescence, Lake Erie, Lake Simcoe, Lake Ontario, PARAFAC

## Introduction

In aquatic ecosystems, dissolved organic matter (DOM) is the largest pool of organic matter and is composed of organic forms of carbon (C), nitrogen, sulphur, and phosphorus (P; Wetzel 1992, Aitkenhead-Peterson et al. 2003). Dissolved organic carbon (DOC) is a dominant component of DOM (~60%), and most analytical methods for measuring organic matter actually determine the C content only. Typically, the DOM pool is composed of 50% humic substances (fulvic and humic acids), 30% macromolecular hydrophilic acids, and 20% low-molecular-weight (LMW) compounds (e.g., carbohydrates, amino acids; Aitkenhead-Peterson et al. 2003), although these

proportions are variable. The humic substances in turn are dominated by fulvic acids, which are yellow, moderate molecular weight organic acids originating from the microbial degradation of plant and animal remains (McKnight et al. 2003).

In freshwater ecosystems, sources of DOM are often dominated by allochthonous inputs from the surrounding catchments, especially in boreal lakes, which are supplemented by autochthonous DOM generated by in-lake processes, and atmospheric inputs (e.g., Laird and Scavia 1990, Huang and Chen 2009). For any given ecosystem, the quantity and quality of DOM will be a balance between external inputs and in-lake processes (production and losses), which are in turn influenced by internal and

external factors such as water residence time, lake trophic status, watershed hydrology and soil composition, land use, and local precipitation (Aitkenhead-Peterson et al. 2003, Huang and Chen 2009, Roehm et al. 2009). Sources of allochthonous DOM are in part the product of atmospheric dust and gases, and leaf and root litter, as well as primary and secondary metabolites of microorganisms (Aitkenhead-Peterson et al. 2003). Sources of autochthonous DOM typically originate from the extracellular or viral release of DOM from algae and macrophytes, the senescence of these organisms, and the production of DOM through grazing (Bertilsson and Jones 2003).

The main in-lake losses of DOM are bacterial utilization (i.e., the microbial loop) and photochemical oxidation (Steinberg 2003). The flow of energy and C in aquatic ecosystems is dictated in part by the activity of the microbial loop, and in turn, the quality (i.e., bioavailability) of the DOM will influence the metabolism and growth of bacteria (Benner 2003). For example, phytoplankton-derived, high-molecular-weight (HMW) DOM fuels high rates of bacterial metabolism while LMW DOM supports high bacterial growth efficiencies (Benner 2003, Bertilsson and Jones 2003). Allochthonous DOM also represents a significant C source for microbes. While some allochthonous DOM is considered directly bioavailable (Fellman et al. 2009), most of the terrestrial DOM is relatively less bioavailable until photochemical reactions oxidize it, generating more suitable substrates for bacterial uptake (Moran and Covert 2003, Jansson et al. 2007). The oxidation of DOM also releases nutrients (nitrogen and P) and produces carbon dioxide (CO<sub>2</sub>), which can influence lake productivity (Moran and Zepp 1997, Moran and Covert 2003).

The various factors that influence the sources and processing of DOM in aquatic systems ultimately play an important role in ecosystem function and structure. DOM quantity and quality have a large influence on the chemistry and biology of aquatic ecosystems; it attenuates visible and ultraviolet radiation, influences nutrient availability, modulates metal availability, and affects the heat budgets of lakes (i.e., stratification; Aitkenhead-Peterson et al. 2003, Mulholland 2003). In this study, we characterized and compared the variability in DOM quantity and quality in Lake Simcoe using parallel factor analysis (PARAFAC) analysis and compared it to lakes Erie and Ontario to further our understanding of the sources, composition, and transformation of DOM in the lower Great Lakes basin.

## Study sites

Lake Erie, Lake Ontario, Hamilton Harbour (an embayment in western Lake Ontario), and Lake Simcoe are located in southern Ontario (Fig. 1). The catchments

draining these lakes vary from highly urbanized (Hamilton Harbour, Lake Ontario) to largely agricultural (Lake Erie), to mixtures of urban, agricultural, and forested landscapes (e.g., Lake Simcoe). Lake Ontario has an area of 18 960 km<sup>2</sup>, a drainage basin area of 64 030 km<sup>2</sup>, an average depth of 86 m, and maximum depth of 244 m. Four nearshore areas were sampled in Lake Ontario, representing a range of land uses: Cobourg, Ajax, Toronto, and Grimsby. Tributaries from each Lake Ontario site were sampled in addition to the lake. Hamilton Harbour, a eutrophic embayment of western Lake Ontario, has an area of 21.6 km<sup>2</sup>, a drainage basin area of 500 km<sup>2</sup>, an average depth of 13 m, and maximum depth of 25 m. Only one station at the center of the harbour was sampled, and no tributaries were sampled. Lake Erie has an area of 25 700 km<sup>2</sup>, a drainage basin area of 78 000 km<sup>2</sup>, an average depth of 19 m, and maximum depth of 64 m. Two nearshore areas were sampled in Lake Erie, both of which have watersheds dominated by agriculture: Grand River and Port Stanley. Within each nearshore area sampled in lakes Ontario and Erie, 20 to 25 stations were sampled. Lake Simcoe has an area of 722 km<sup>2</sup>, a drainage basin area of 2899 km<sup>2</sup>, an average depth of 14 m, and maximum depth of 42 m. Four areas were sampled in Lake Simcoe: Kempenfelt Bay, Cook's Bay, Main basin, and near the outflow of the lake; tributaries were also sampled along these 4 areas. In Lake Simcoe, 13 lake stations and 7 tributaries were sampled: Beaverton Creek, Black River, East Holland River, Hawkestone Creek, Lovers Creek, Pefferlaw Brook, and Whites Creek. In addition, 4 wastewater treatment plant effluents (Keswick, Barrie, Orillia, and Sutton) and 4 beach groundwater sites (Balfour, De LaSalle, Mara, and Minet Point beaches) were sampled.

## Methods

### Sample collection and chemical analysis

Lake Ontario and its tributaries were sampled in 2008, and Lake Erie, Hamilton Harbour, and Lake Simcoe and its tributaries were sampled in 2009, all between April and October. The seasons were operationally defined as (1) spring: April and May; (2) Summer: June, July, and August; and (3) fall: September and October. Water samples were taken using a Van Dorn water sampler (in all lakes and the Holland River) or by wading into the water and filling a sampling bottle (all other tributaries). Lake and Holland River samples were collected at 1 m depth, while all other tributary samples were taken at 10 cm below or at the surface. Discrete water samples were kept in a cooler in the dark while in the field and refrigerated until analysis in the laboratory. In addition,

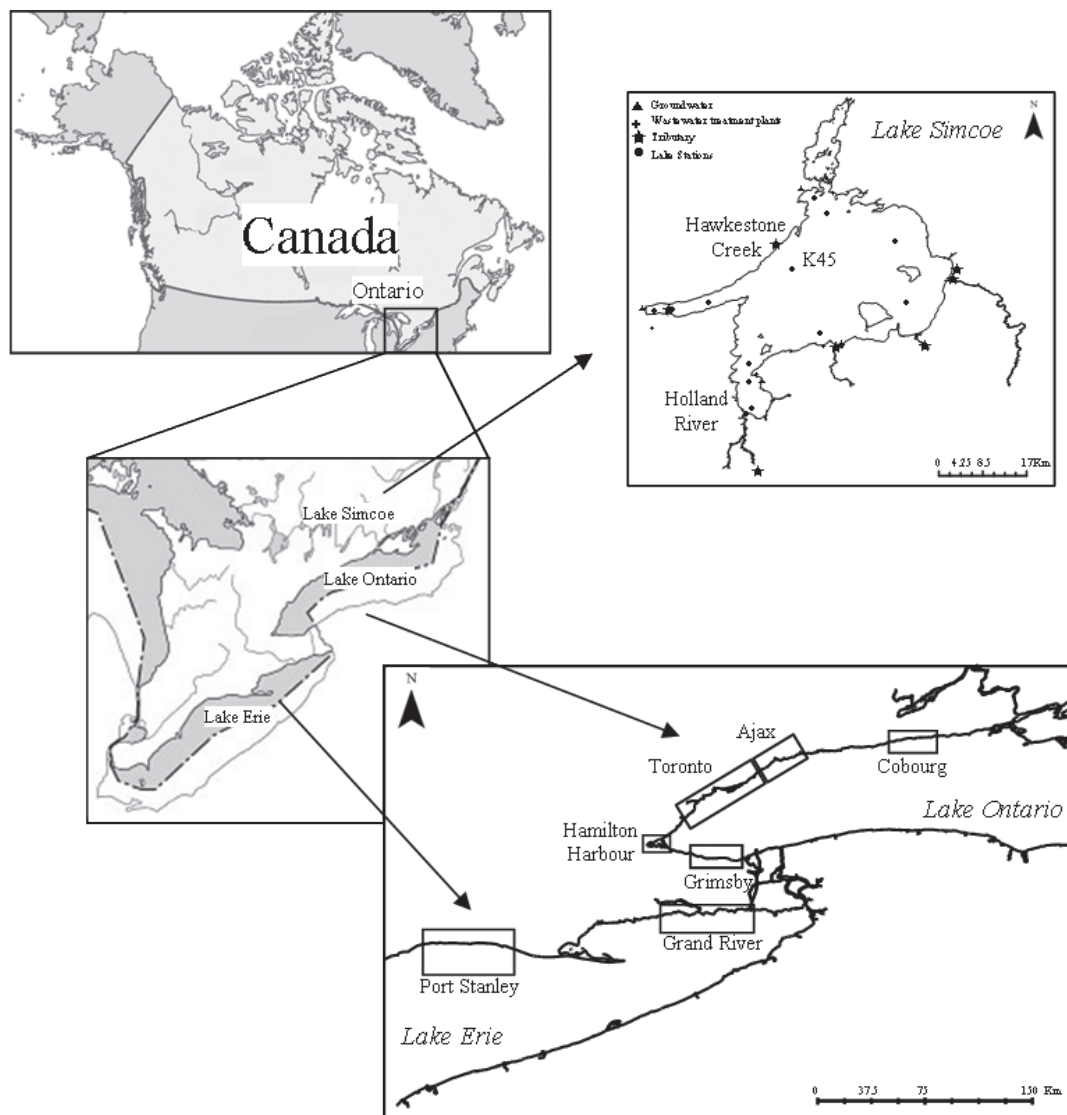
water column profiles (YSI 6600, Hoskin Scientific, Burlington, ON; XRX-420, RBR Ltd, Ottawa, ON) were also conducted at lake stations where discrete water samples were collected to record conductivity levels.

Discrete epilimnetic water samples were analyzed for a variety of water chemistry parameters, including DOM, total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate/nitrite ( $\text{NO}_{3/2}$ ), total ammonia ( $\text{NH}_3$ ), and chlorophyll *a* (Chl-*a*) concentrations. Concentrations of DOM, reported as C content, were determined using a UV-persulfate TOC analyzer (Pheonix 8000™). Briefly, filtered (0.45  $\mu\text{m}$ ) water samples were acidified with phosphoric acid to pH of 3 or lower; mixed with sodium persulfate

and phosphoric acid solutions in a UV reactor chamber; and the  $\text{CO}_2$  produced was sent to a nondispersive infrared (NDIR) detector to calculate DOC concentrations in  $\text{mg C L}^{-1}$ . The analytical methods for all other parameters have been described elsewhere (Hiriart-Baer et al. 2009).

### Absorbance measurements

Total coloured DOM (C-DOM) absorption was measured spectrophotometrically on all samples, except Lake Ontario, after filtration through 0.45  $\mu\text{m}$  membrane filters. Absorbance, or optical density, of the filtrate was measured using a dual beam VARIAN Cary-300 spectro-



**Fig. 1.** Map of the 3 lakes sampled for this study, all located in southern Ontario, Canada. The Lake Simcoe map shows the location of lake (circles), tributary (stars), groundwater (triangles), and wastewater treatment plant (crosses) sites as well as the 4 main areas sampled in Lake Simcoe (Kempfenfelt Bay, Cook's Bay, Main basin, and near the outflow of the lake). Included also are the locations of the DOM sample end members: Hawkestone Creek, Holland River, the 4 wastewater treatment plants, and K45 (see text for further details). On the lakes Ontario and Erie map, boxes delineate the 6 areas sampled for these lakes in addition to Hamilton Harbour, at its most western tip.

photometer, scanning from 800–200 nm at 1 nm increments. Distilled water at the same ambient temperature as the sample was used as a reference. The spectral absorption coefficient,  $a_{\text{CDOM}}(\lambda)$ , was calculated from the measured optical density of the sample ( $\text{OD}_{\text{sl}}(\lambda)$ ) according to:

$$a_{\text{CDOM}}(\lambda) = \frac{2.303}{l} [\text{OD}_{\text{sl}}(\lambda) - \text{OD}_{(\text{null})}], \quad (1)$$

where  $l$  is the cuvette pathlength (0.01 m) and  $\text{OD}_{(\text{null})}$  is the optical density at a wavelength where absorption by C-DOM can be assumed to be zero: in this case, the average over 740–750 nm. Spectra displaying anomalous negative absorption in the visible range were removed from the analysis. DOM-specific absorption coefficients  $A^*(\lambda)$  were calculated as  $a(\lambda)/(\text{DOC})$ . Specifically,  $A^*_{254}$  was calculated and used as an indicator of DOM aromaticity with higher  $A^*_{254}$  values indicative of more aromatic DOM (Weishaar et al. 2003). The E2:E3 ratio calculated as the ratio of  $a_{254}:a_{365}$  was used as a surrogate for DOM average polarity, with relatively lower ratios representing, on average, DOM with relatively higher molecular weight (De Haan and De Boer 1987). The spectral slope ( $S$ ) for the intervals 275–295 nm and 350–400 nm as calculated by fitting the absorption spectra to a single exponential decay function (equation 2). The exponential function,  $S$ , describes the spectral absorption slope between  $\lambda$  and  $\lambda_0$  and was calculated here between  $a_{275}$  and  $a_{295}$ :

$$a_{(\lambda)} = a_{(\lambda_0)} e^{[-S(\lambda-\lambda_0)]}. \quad (2)$$

The slope ratio ( $S_R$ ) was then calculated as the ratio of  $S_{275-295}/S_{350-400}$  and its change over time was used as an indicator of whether photooxidation or microbial decomposition dominated DOM degradation (Helms et al. 2008).

### Fluorescence excitation-emission matrices

Fluorescence excitation and emission matrices (EEMs) of DOM were performed on filtered (0.45  $\mu\text{m}$ ) water samples using a Fluor-Imager M53 (Skalar, Netherlands) with a 150 W Xe-lamp. The scanning wavelength ranges used were 240–360 nm for excitation and 260–575 nm for emission wavelengths, with a 20 nm shift between excitation and emission wavelengths. Fluorescence reading intervals were 5 nm for both excitation and emission. Readings on the Fluor-Imager were not collected in ratio mode, thus uncorrected spectra were divided by an excitation correction file generated per the manufacturer (Skalar). No emission correction was available from the manufacturer; thus no correction was applied.

Each EEM was Raman calibrated (Stedmon et al.

2003) and further corrected for inner filter effects (except EEMs for Lake Ontario and its tributaries; McKnight et al. 2001) and Raman scatter (Milli-Q water blank subtraction; Stedmon and Bro 2008). Because Lake Ontario samples did not include absorbance measurements, no inner filter correction could be applied; however, Lake Ontario absorbance values of  $\sim 5 \text{ m}^{-1}$  at 240 nm (estimated from exponential slope extrapolation [300–750 nm scans]; C. Binding, Environment Canada, Research Scientist, pers. comm.) were measured in 2008 and as such, inner filter effects are likely to be negligible for the lake water samples (Stedmon and Bro 2008). Because Lake Ontario tributary samples, which are expected to have non-negligible inner filter effects, could not be corrected, these samples were excluded from the PARAFAC analysis.

The EEMs were then imported into MATLAB and processed using the MATLAB dissolved organic matter fluorescence (DOM-Fluor) toolbox, which included the removal of any residual Raleigh scatter (Stedmon and Bro 2008). A total of 1604 EEMs were measured; 21 were removed after visual inspection of the datasets, suggesting that these samples either contained measurement errors or they were deemed outliers following the outlier testing of the DOM-Fluor toolbox. Of the remaining 1583 EEMs, statistical deconvolution of the spectra and component characterization was performed through PARAFAC, including split-half model validation to confirm the number of fluorescent components characterized in the samples and randomization of the starting parameter values to optimize the derived model (Stedmon and Bro 2008). The fluorescence intensity of each component is the  $F_{\text{max}}$  output value of the PARAFAC analysis. C-DOM was then calculated as the sum of  $F_{\text{max}}$  for all components for each sample.

### Data processing

All data were tested for normality and  $\log_{10}$  transformed where necessary. Treatment (seasons) and sample type (i.e., body of water) effects were tested by analysis of variance (ANOVA) with the Tukey post hoc hypothesis testing of significant differences. The statistical package used for all analyses was SYSTAT version 12 (Systat Software Inc., Chicago, Illinois, USA). Significance was determined at a  $p$  value of 0.05.

## Results

### Water quality conditions

Based on TP median surface water concentrations, the average trophic status of the 4 different water bodies in this study varied from oligotrophic to meso-eutrophic (Table 1). Even in systems considered oligotrophic,



however, nutrient and Chl-*a* concentrations can reach eutrophic levels (e.g., Lake Ontario). These eutrophic conditions were neither ubiquitous nor restricted to shallow (<2 m) waters, suggesting areas where watershed material transfer is higher (data not shown).

### DOC and C-DOM

Median concentrations of DOC ranged from 2.0 to 4.4 mg C L<sup>-1</sup> between lakes, with Lake Simcoe having the highest median concentrations and maximum values observed in surface waters as well as the highest tributary concentrations (Fig. 2; Table 1). Similar to nutrient and Chl-*a* concentrations, high DOC concentrations were observed within study lakes, suggesting areas of higher watershed material transfer (Table 1). In general, there was a good correlation between DOC concentrations and conductivity levels with the possible exception of Lake Simcoe (Fig. 2). Similar patterns were observed between DOC and TP concentrations (data not shown). In Lake Simcoe, for each unit of TP or conductivity measured in lake water there were twice as many units of DOC; however, there was a similar correlation between C-DOM, reported as total component  $F_{max}$ , and conductivity for all lakes (Fig. 2).

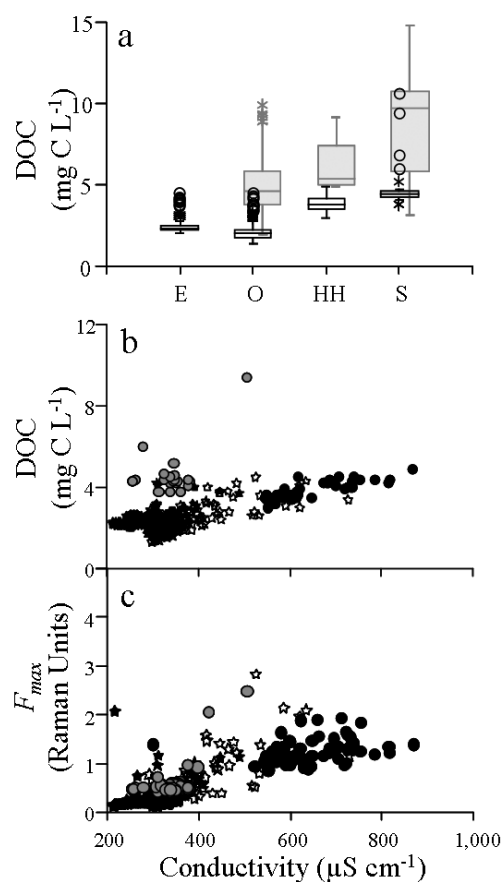
Spatial and temporal differences in lake water DOC and C-DOM were observed between all 4 study areas (Table 2). On average, DOC concentrations and C-DOM fluorescence intensities were lowest in lakes Erie and Ontario and highest in Lake Simcoe and Hamilton Harbour (Table 2). Similarly, values were highest in the spring and lowest in the fall for all systems. C-DOM/DOC ratios followed the same spatial pattern but showed no significant seasonal differences (Table 2). In Lake Simcoe, DOC, C-DOM, and C-DOM/DOC ratios differed between sample types (Table 3). On average, lake water samples were significantly lower and wastewater treatment plant samples were significantly higher than tributary and groundwater samples, which did not always differ significantly from each other (Table 3). Seasonal differences were only observed for DOC and, in turn, only for groundwater samples with significantly lower DOC concentrations in the spring.

Seasonal patterns in DOC were observed in lakes Erie and Ontario, including Hamilton Harbour (Table 2). Lake Erie and Hamilton Harbour showed significant decreases in DOC from spring to fall, while Lake Ontario showed a significantly higher summer DOC concentration. There seems to be a net loss of DOC from spring to fall in Lake Erie and Hamilton Harbour, while there seems to be no net change in DOC concentrations in lakes Ontario and Simcoe during this time. Similarly, Lake Ontario showed a significant increase in C-DOM in the summer while

Hamilton Harbour showed a significant decrease from spring to summer (Table 2).

### DOM characterization

In this large dataset, 5 components were identified using PARAFAC modeling (Fig. 3). The 5-component model obtained was validated through split-half analysis. The validation is evident from the consistency of both the excitation and emissions loadings derived for each analyzed split of the dataset (Fig. 3a–f). The 5 components identified in this dataset showed similarities with PARAFAC components identified in earlier studies



**Fig. 2.** DOC concentrations (a) in lake water (black) and tributaries (gray) sampled in the 4 study areas. Relationship between total DOC concentrations (b) and  $F_{max}$  (c) with conductivity in surface (1 m) lake water samples for lakes Erie (E; filled stars), Ontario (O; open stars), Simcoe (S; gray circles), and Hamilton Harbour (HH; black circles). Hamilton Harbour tributary DOC concentrations (provided by the Ontario Ministry of the Environment [T. Labencki, Ontario Ministry of the Environment, 2010–2012 samples, unpubl. data]). The length of the box plots shows the range that includes 50% of the data values, with the box edges (i.e., hinges) at the first and third quartile; the error bars represent 1.5 times the interquartile range; values below 3 times the interquartile range are plotted as asterisks; and values beyond that are plotted as circles.

**Table 1.** Limnological parameters measured in the lake surface waters and tributaries of lakes Erie, Ontario, Simcoe, and Hamilton Harbour. Values represent the median and the ranges are in parentheses. Trophic status (O: oligotrophic; O-M: oligo-mesotrophic; M: mesotrophic; M-E: meso-eutrophic) was derived based on TP concentrations (Wetzel 2001). Subscripts denote the sample size for each parameter.

Limnological parameters	Erie	Ontario		Hamilton Harbour	Simcoe	
	Lake	Lake	Tributaries	Lake	Lake	Tributaries
Total phosphorus ( $\mu\text{g L}^{-1}$ )	10.7 <sub>121</sub> (5.3–81.1)	7.0 <sub>762</sub> (0.5–370.0)	55.0 <sub>120</sub> (8.0–510.0)	41.5 <sub>61</sub> (24.0–94.1)	14.8 <sub>32</sub> (8.7–125.0)	32.4 <sub>9</sub> (9.9–73.8)
Soluble reactive phosphorus ( $\mu\text{g L}^{-1}$ )	1.2 <sub>121</sub> (0.3–10.1)	0.6 <sub>735</sub> (0.1–291.0)	4.8 <sub>120</sub> (0.5–247.0)	2.2 <sub>62</sub> (1.2–37.6)	6.7 <sub>35</sub> (0.1–76.7)	25.7 <sub>9</sub> (2.1–212.2)
Nitrate/Nitrite ( $\mu\text{g L}^{-1}$ )	201 <sub>57</sub> (103–3520)	399 <sub>764</sub> (7–2890)	629 <sub>119</sub> (5–2230)	1935 <sub>62</sub> (1240–2890)	n.a.	n.a.
Ammonia ( $\mu\text{g}^{-1}$ )	12 <sub>121</sub> (0–48)	22 <sub>764</sub> (2–3670)	20 <sub>120</sub> (2–4550)	35 <sub>62</sub> (5–785)	n.a.	n.a.
Chlorophyll <i>a</i> ( $\mu\text{g L}^{-1}$ )	1.2 <sub>64</sub> (0.2–11.2)	2.0 <sub>592</sub> (1.0–35.0)	3.6 <sub>109</sub> (1.0–102.0)	12.1 <sub>61</sub> (0.7–40.9)	1.2 <sub>29</sub> (0.3–57.8)	4.4 <sub>6</sub> (0.3–5.7)
Dissolved organic carbon ( $\text{mg L}^{-1}$ )	2.3 <sub>121</sub> (2.0–4.5)	2.07 <sub>60</sub> (1.3–4.5)	4.3 <sub>122</sub> (1.7–9.9)	3.8 <sub>52</sub> (3.0–4.9)	4.3 <sub>34</sub> (3.8–10.6)	7.0 <sub>9</sub> (4.3–14.8)
Conductivity ( $\mu\text{S cm}^{-1}$ )	263 <sub>100</sub> (213–489)	3117 <sub>60</sub> (289–728)	561 <sub>122</sub> (297–2360)	615 <sub>86</sub> (545–869)	341 <sub>63</sub> (249–506)	n.a.
Secchi (m)	3.0 <sub>80</sub> (0.3–10.5)	4.0 <sub>401</sub> (0.2–24.0)	n.a.	2 <sub>44</sub> (1.3–3.5)	n.a.	n.a.
Trophic status	O–M	O	M–E	E	O–M	M

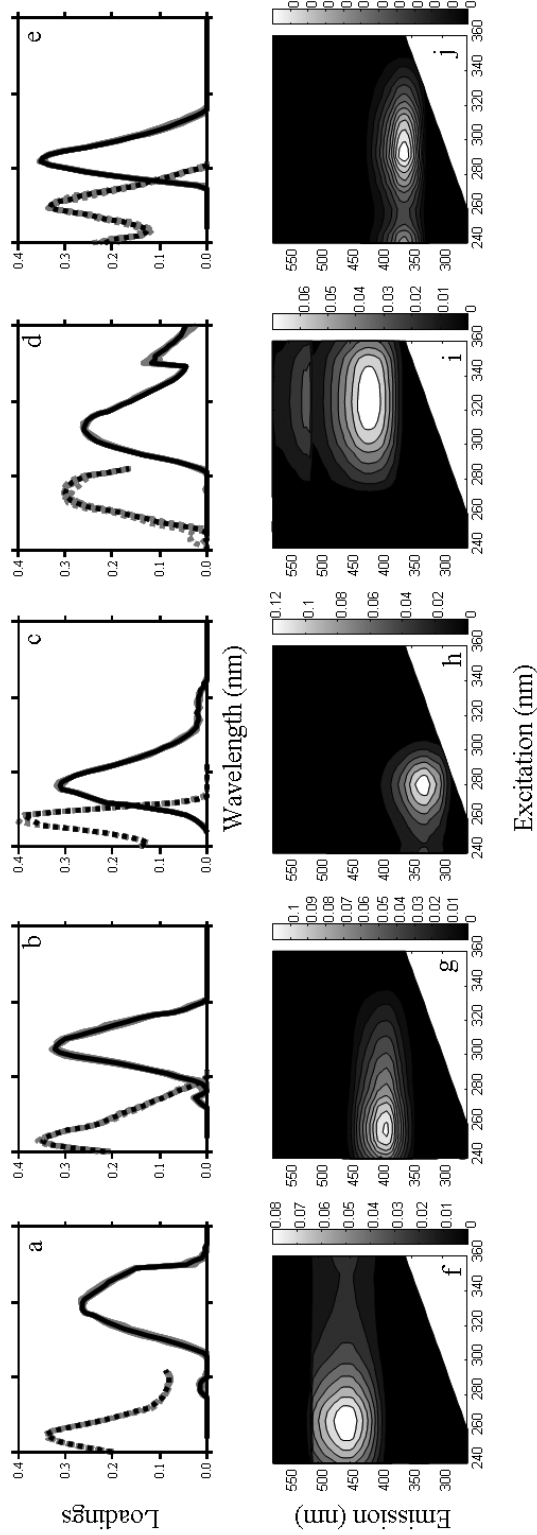
(Table 4). Components 1, 2, and 4 were identified as terrestrially derived humic matter fluorophores, which would correspond to previously identified peaks A ( $\alpha'$ ) and M (Coble 2007). Components 1, 2, and 4 were well correlated to each other (Pearson coefficients,  $0.92 < r < 0.97$ ), consistent with the suggested sources of the components (Table 4). Components 1, 2, and 4 are terrestrial in origin and are likely delivered to the systems through similar mechanisms (e.g., tributaries). Components 3 and 5 are representative of peaks T ( $\delta$ ) and N, respectively (Coble 2007), which are both thought to be related to biological production (Table 4). Components 3 and 5 showed a stronger correlation to each other ( $r > 0.95$ ) than with any other component ( $0.55 < r < 0.84$ ), suggesting that the sources of both components 3 and 5 are likely autochthonous.

The distribution of the 5 PARAFAC components showed different distributions among the 4 end members identified in Lake Simcoe (Fig. 4). Component 1 showed high fluorescence in all end members and clearly dominated in both stream end members. The agricultural stream showed higher fluorescence of components 3 and 5 than the natural heritage stream. These components were also important in the wastewater end member, while components 1, 2, and 3 dominated the open water samples. The relative distribution of all these components among the different end members is consistent with their suggested origins.

The relative abundance of all 5 components was similar among lakes with the possible exception of Lake Simcoe (Fig. 5). Components 1, 2, and 3 dominated in lake (open) water over components 4 and 5 (Fig. 4). On

**Table 2.** Two-way ANOVA statistics, testing for spatial and seasonal (Sp = Spring; S = Summer; F = Fall) differences in DOC concentrations, C-DOM fluorescence intensities, and C-DOM/DOC ratios in lakes Erie (E), Ontario (O), Simcoe (S), and Hamilton Harbour (HH). Paired significance testing was done using the post-hoc Tukey test. Different superscripts denote significant differences ( $p < 0.05$ ) between lakes or seasons, while  $<$  or  $>$  represent values less than or greater than each other, respectively.

Parameter	Lake	Post-hoc test	Seasonal	Post-hoc test	Lake x Season	Post-hoc test
$\log_e$ DOC	$F_{3,761}=337.3, p<0.001$	$O^a < E^b < HH^c < S^d$	$F_{2,761}=10.6, p<0.001$	$Sp^a = S^a > F^b$	$F_{6,761}=6.6, p<0.001$	$O-Sp^a < S^b > F^a$ $E-Sp^d = S^a > F^b$ $S-Sp^a = S^a = F^a$ $HH-Sp^a > S^b > F^c$
$\log_e$ C-DOM	$F_{3,871}=190.2, p<0.001$	$O^a = E^a < S^b < HH^c$	$F_{2,871}=3.4, p=0.032$	$Sp^a = S^{ab} = F^b$	$F_{6,871}=3.3, p=0.003$	$O-Sp^{ab} = S^a > F^b$ $E-Sp^d = S^a = F^a$ $S-Sp^a = S^a = F^a$ $HH-Sp^a = S^b = F^a$
$\log_e$ C-DOM/DOC	$F_{3,761}=65.5, p<0.001$	$O^a < E^b = S^b < HH^c$	$F_{2,761}=0.1, p=0.906$	—	$F_{6,761}=0.2, p=0.974$	—



**Fig. 3.** Component loading spectra (a–e) for excitation (dotted lines) and emission (solid lines) wavelengths derived using PARAFAC analysis and excitation emission matrices for the 5 components identified (f–j; components 1 to 5 from left to right). The thick gray lines represent the results of the split-half model validation analysis and the thick black lines represent the model using the entire dataset.

**Table 3.** Two-way ANOVA statistics testing for differences in DOC, C-DOM, and C-DOM/DOC ratios between sample types (L = lake; GW = groundwater; T = Tributary; WW = wastewater) collected in Lake Simcoe. Paired significance testing was done using the post-hoc Tukey test. Superscripts denote significant differences ( $p < 0.05$ ) between sample types and seasons, while  $<$  or  $>$  represent values less than or greater than each other, respectively.

Parameter	Sample type	Post-hoc test	Seasonal	Post-hoc test	Type x Season	Post-hoc test
$\log_{10}$ DOC	$F_{3,46}=16.5, p<0.001$	$L^a = GW^a < T^b = WW^b$	$F_{2,46}=4.7, p=0.014$	$Sp^a < S^b = F^b$	$F_{6,46}=4.3, p=0.002$	$L-Sp^a = S^a = F^a$ $GW-Sp^a < S^b = F^b$ $T-Sp^a = S^a = F^a$ $WW-Sp^a = S^a = F^a$
$\log_{10}$ C-DOM	$F_{3,53}=26.6, p<0.001$	$L^a = GW^a < T^b = WW^b$	$F_{2,53}=0.5, p=0.618$	—	$F_{6,53}=1.2, p=0.336$	—
$\log_{10}$ C-DOM/DOC	$F_{3,46}=56.6, p<0.001$	$L^a < GW^b = T^b = WW^c$	$F_{2,46}=1.4, p=0.264$	—	$F_{6,46}=1.9, p=0.101$	—

average, all components were lowest in lakes Erie and Ontario, higher in Lake Simcoe, and highest in Hamilton Harbour. Using the differences in the end member signatures, we attempted to evaluate some of the DOM source differences between the lakes (Fig. 6b). Looking at the ratio of components 2 (agricultural origin) and 5 (autochthonous origin) to component 1 (terrestrial origin ubiquitous to all samples; Table 4), the data suggest that Hamilton Harbour and Lake Ontario have significantly ( $p < 0.005$ ) higher relative contributions of wastewater derived DOM than either lakes Erie or Simcoe, while Lake Simcoe has a significantly ( $p < 0.005$ ) higher relative contribution of wastewater derived DOM than Lake Erie. Relative contributions of the agriculturally derived DOM to the systems were not evident from this data. While Lake Simcoe showed slightly higher fluorescence intensities of component 2 relative to lakes Erie and Ontario, fluorescence intensities of component 2 were significantly higher in Hamilton Harbour compared to all lakes.

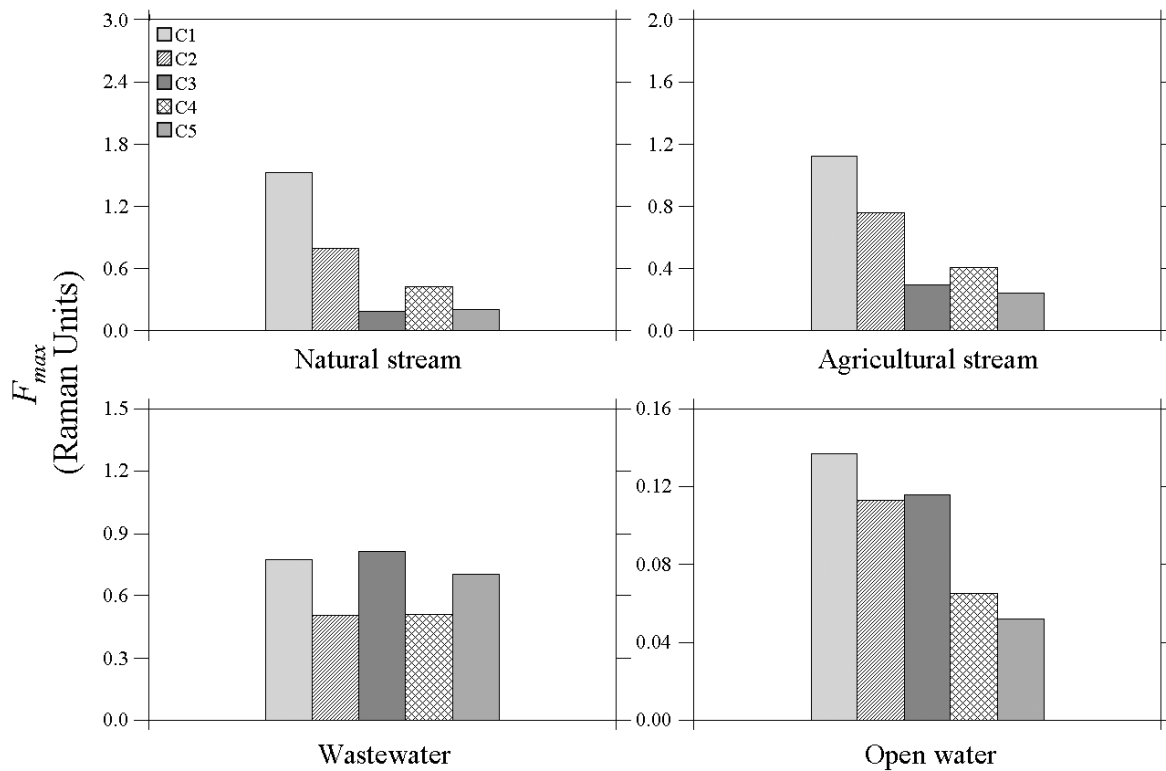
DOM transformation processes were investigated with 3 optical parameters, the spectral slope ratio term ( $S_R$ ), E2:E3 ratio, and  $A^*_{254}$ , as indicators of dominant transformation processes, average molecular weight, and aromaticity, respectively. In Lake Erie,  $A^*_{254}$  was significantly ( $p < 0.05$ ) higher in the spring compared to the summer, and in Lake Simcoe  $A^*_{254}$  decreased significantly ( $p < 0.005$ ) between summer and fall. No significant changes were observed in Hamilton Harbour. E2:E3 increased significantly ( $p < 0.05$ ) from spring to fall in Lake Erie, while in Lake Simcoe a significant ( $p < 0.05$ ) decrease from summer to fall was observed (Fig. 7). There was no significant change in the  $S_R$  parameter for Lake Erie or Hamilton Harbour, while Lake Simcoe ( $p < 0.005$ ) showed a significant increase in  $S_R$  from spring to summer followed by no change into the fall.

## Discussion

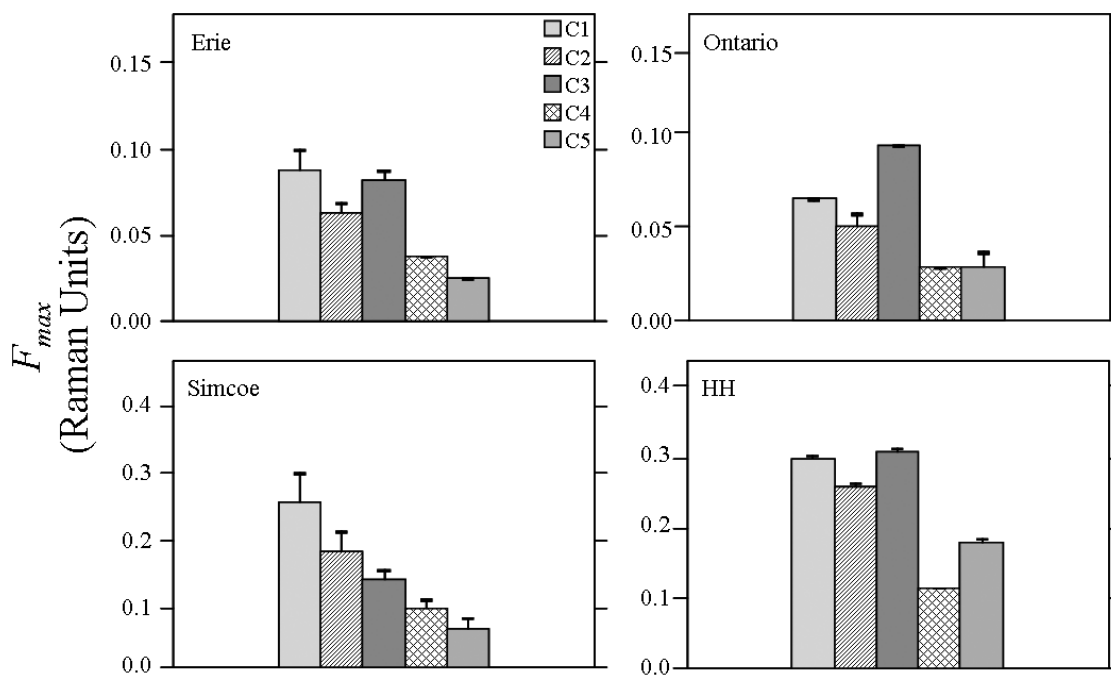
### DOM quantity and quality

The highest concentrations of DOC were measured in Lake Simcoe and its tributaries, which were higher than those measured in the heavily impacted Hamilton Harbour and its tributaries. The relationships between DOC, C-DOM, and conductivity for lakes Erie and Ontario and Hamilton Harbour suggest that both DOC concentrations and C-DOM fluorescence intensity are related in large part to watershed material transfer. This is commonly observed in many freshwater ecosystems, whereby watershed inputs of DOM often dominate (Wetzel 2001). Lake Simcoe showed a different relationship, however, where more than expected DOC, and to a certain extent C-DOM, was





**Fig. 4.** Relative fluorescence intensities of the 5 PARAFAC components measured as  $F_{max}$  in the 4 identified end members in the Lake Simcoe watershed. Natural Stream: Hawkestone Creek; Agricultural Stream: West Holland River; Wastewater: Barrie, Orillia, Sutton and Keswick wastewater treatment plant effluent; Open water: Main basin deep station (K45).



**Fig. 5.** Relative fluorescence intensities of the 5 PARAFAC components measured as  $F_{max}$  in lakes Erie, Ontario, Simcoe, and Hamilton Harbour (HH). Note the difference in scales for the individual components. Error bars are bidirectional, but for clarity only the positive error bar is presented.

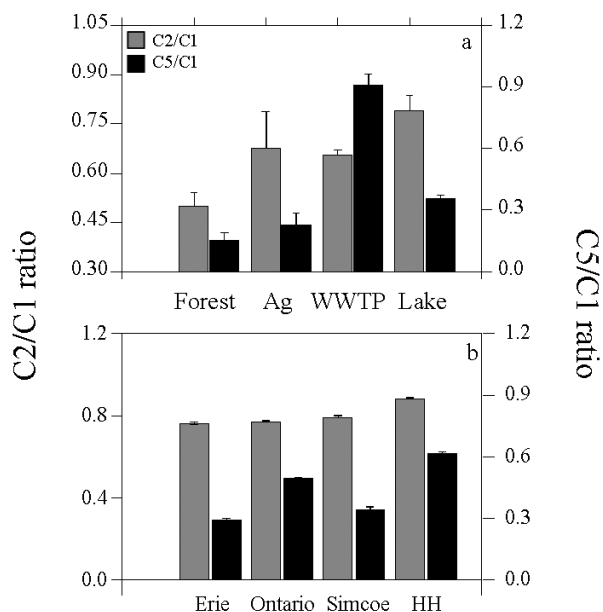
**Table 4.** Position of the fluorescence maxima of the 5 components identified by PARAFAC analysis, their comparison to previously identified components and previously reported fluorophore peaks, and their tentative origins. References: a. Stedmon and Markager 2005; b. Fellman et al. 2008; c. Williams et al. 2010; d. Murphy et al. 2008; e. Lapiere and Frenette 2009.

Component	Ex/Em maxima	References for previously identified components					Peaks*	Sources
		a	b	c	d	e		
1	265(>360)/460	4	3		3		A	Ubiquitous, terrestrial fulvic acid group
2	255/390	6			8	5	A	Terrestrial, humic substances, possibly agricultural DOM related to manure spreading on fields
3	280/330	7	8		6	8	T	Autochthonous, tryptophan-like, amino acids
4	325/420	5			2	7	M	Marine-like or terrestrial humic matter, possibly of anthropogenic and/or agricultural origin
5	290(<240)/360			5	7		N	Autochthonous, protein-like, related to biological production, microbially produced

\*Coble 2007

observed based on conductivity and TP concentrations, suggesting that Lake Simcoe has another important source of DOM likely independent of watershed material transfer.

Two groups of DOM components were identified with PARAFAC; 3 allochthonous-derived (components 1, 2, and 4) and 2 autochthonous-derived DOM (components 3 and 5) components. The distribution of the terrestrially derived components between lakes sampled suggests that



**Fig. 6.** Ratios of components 2 and 5 to component 1 in (a) the 4 end members identified in the Lake Simcoe watershed; and (b) lakes Erie, Ontario, Simcoe, and Hamilton Harbour (HH). The C2/C1 ratio is used as an agricultural tracer while the C5/C1 ratio is used as a wastewater effluent tracer; see text for further details. See Fig. 4 caption for details. Note the difference in scales. Error bars are bidirectional, but for clarity only the positive error bar is presented.

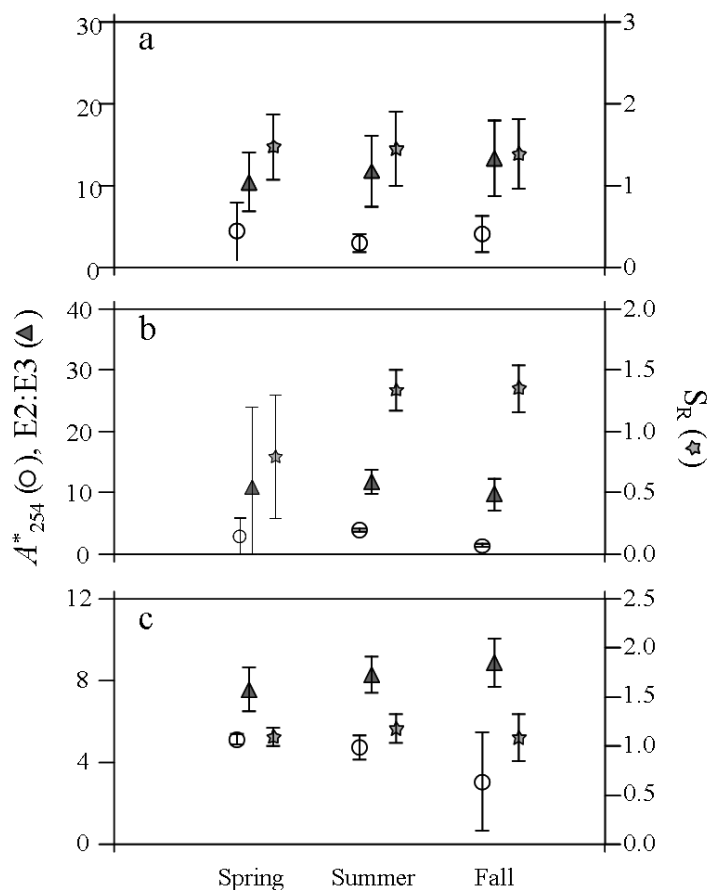
Hamilton Harbour receives the largest amount of allochthonous C-DOM, while lakes Erie and Ontario receive the least. This is consistent with the ratio of watershed:lake surface area for lakes Erie, Ontario, Simcoe, and Hamilton Harbour (3.0, 3.4, 4.0, and 23.1, respectively), although Hamilton Harbour seems to receive a disproportionately smaller amount of C-DOM than the rest of the lakes. This may be a consequence of the urban land use (28%; Bowlby et al. 2009) in this catchment, located largely within the immediate surrounding area of the harbour, and the presence of 6 reservoirs along its 2 main tributaries (Spencer and Grindstone Creeks). These reservoirs may allow increased processing and transformation of allochthonous DOM because they allow longer hydrologic residence times (Mash et al. 2004).

The distribution of the autochthonous components between the lakes sampled was not unexpected; the most nutrient enriched system showed the highest fluorescence intensity for components 3 and 5. These components, similar to peaks T and N, respectively, reviewed by Coble (2007), have been associated with biological production, and highest relative concentrations are typically observed during the summer months (Stedmon and Markager 2005). Summer increases in component 3 were observed in Lake Ontario but were not observed in lakes Erie and Simcoe and Hamilton Harbour. The absence of an increase in Lake Simcoe is unclear given the relatively higher TP concentrations (albeit still low) compared to Lake Ontario and higher water clarity (Young et al. 2010). In Hamilton Harbour, however, nutrients may not be the sole controlling factor of biological production; other factors such as light may also be important in this ecosystem (Hiriart-Baer et al. 2009). Additionally, higher nutrient concentrations in Hamilton Harbour may be supporting a

higher bacterial utilization of component 3, a labile fraction of the C-DOM pool (Baker and Inverarity 2004), which may be contributing to the observed net decrease in this component. The proportion of tyrosine and tryptophan-like DOM has been correlated to the proportion of DOM loss in laboratory incubations (Balcarczyk 2008), and in Hamilton Harbour, the hypolimnetic oxygen depletion rate is highly correlated ( $p < 0.01$ ,  $r^2 = 0.71$ ) to spring DOC concentrations (V. Hiriart-Baer, Environment Canada, Research Scientist, March 2012, unpubl. data) when relative fluorescence intensities of component 3 are highest (data not shown).

Autochthonous DOM, derived mainly from phytoplankton and macrophytes, can be an important contributor to DOC in aquatic ecosystems. For example, the seagrass *Thalassia testudinum*, which is widespread in the Florida everglades, and macrophytes and their epiphytes in Lake Saint-Pierre, Quebec, are thought to contribute significantly to the C-DOM pool in these systems (Stabenau et al. 2004, Lapierre and Frenette 2009). Macrophyte production is widespread in the nearshore waters of Lake Simcoe; in particular, Cook's

Bay supports a large macrophyte and phytoplankton biomass (Eimers et al. 2005, Depew et al. 2011, Ginn 2011). Cook's Bay had the highest DOC concentrations ( $6.1 \pm 0.8 \text{ mg L}^{-1}$ ) and C-DOM fluorescence intensities ( $1.37 \pm 0.35$  Raman Units [RU]) relative to all other areas sampled in Lake Simcoe (Kempfenfelt:  $4.2 \pm 0.1 \text{ mg L}^{-1}$  and  $0.53 \pm 0.01$  RU; Main:  $4.4 \pm 0.0 \text{ mg L}^{-1}$ , and  $0.54 \pm 0.02$  RU; near outflow of the lake:  $4.5 \pm 0.5 \text{ mg L}^{-1}$  and  $0.51 \pm 0.01$  RU), which is not unexpected because this basin receives ~50% of the watershed inputs to Lake Simcoe (Johnson and Nicholls 1989). Similarly, the eastern basin of Lake Erie has a large proliferation of the macroalgae *Cladophora* in the nearshore (Higgins et al. 2005) which may be contributing, in part, to the higher C-DOM fluorescence intensities and DOC concentrations in the eastern ( $0.35 \pm 0.04$  RU and  $2.6 \pm 0.1 \text{ mg L}^{-1}$ , respectively) compared to the central ( $0.24 \pm 0.04$  RU and  $2.3 \pm 0.1 \text{ mg L}^{-1}$ , respectively) basins sampled. In Lake Simcoe, the large potential pool of autochthonous DOM coupled with its long water residence time (i.e., 11 yr) may lead to an accumulation of autochthonous C-DOM in this system (Mash et al. 2004). The role of DOM on the



**Fig. 7.** Seasonal variability in DOM optical characteristics in lakes (a) Erie, (b) Simcoe, and (c) Hamilton Harbour. Error bars are standard errors of the mean. No absorbance data were available for Lake Ontario.

dissolved oxygen (DO) dynamics in Lake Simcoe is unknown. Although our data were not designed to capture the large pulse of decaying macrophytes, and there was no evidence for it in our fall data, we suggest that a large pulse of DOM following macrophyte senescence, coupled with a long residence time, may be an important component in the hypolimnetic DO cycle of this lake.

### DOM optical properties

DOM transformation processes inferred from changes in the optical properties of C-DOM (i.e.,  $S_R$ ,  $A^*_{254}$ , and E2:E3) differed spatially among lakes and temporally within lakes, with the most pronounced seasonal changes observed in Lake Simcoe. On average, an influx of DOM with higher aromaticity (high  $A^*_{254}$ ) and higher molecular weight (low E2:E3) was observed in the spring across lakes, likely a result of the snow melt flushing the soil organic matter, rich in lignin, that accumulated over the winter months (McKnight et al. 2003, Mulholland 2003). The higher  $A^*_{254}$  observed in the spring decreased in the summer with a concurrent increase in E2:E3 parameters. These changes suggest that C-DOM processing, be it photobleaching or microbial degradation, is shifting the DOM mixture toward smaller and less aromatic compounds (e.g., Helms et al. 2008). Changes in the  $A^*_{254}$  and the E2:E3 parameters were not clearly evident in Lake Simcoe between the spring and summer seasons; the high variability in the spring may be masking any changes in DOM optical properties that would otherwise be observed. The degree of change in DOM quality varied between lakes. For example, the average  $S_R$  parameter in Lake Erie showed no increase between spring and summer while Lake Simcoe showed a significant increase, as would be expected if photobleaching was dominating DOM degradation processes (Helms et al. 2008). A lack of response of the  $S_R$  in Lake Erie could be due to (1) mixing with more photobleached or aged DOM from the open waters of this large lake with already higher spectral slope; and/or (2) smaller terrestrial inputs relative to other lakes (Clark et al. 2009). On average, DOM aromaticity and average molecular weight were lower in Lake Erie, which are indicators of aged and largely processed DOM.

In the transition from summer to fall there seemed to be no consistent pattern in the optical parameters between lakes. In particular, the data suggest that while aromaticity seemed to be reduced, an increase in the average molecular weight in the fall (decrease in E2:E3), which was only observed in Lake Simcoe, suggests that microbial processes may be playing a more important role during this period. Microbial degradation of DOM can lead to the production of higher molecular weight humic substances (Hur 2011). Similarly, a disproportionate

uptake of LMW (and bioavailable) DOM may be shifting the average DOM molecular size (Young et al. 2004). These changes in proposed dominant DOM degradation processes are consistent with a large influx of autochthonous low-colour C-DOM from senescing macrophytes. Alternatively, because seasonal variations in the degree of DOM colour do exist, it is possible that an influx of fresh C-DOM from the watershed, lower in colour and with higher molecular weight, may be contributing to these parameter shifts (Molot and Dillon 1997).

In conclusion, this study showed that both the quantity and quality of the DOM pool can be quite variable among systems over space and time. As expected, autochthonous DOM was related to the productivity of the systems, with Hamilton Harbour showing the highest fluorescence intensities of components 3 and 5, followed by Lake Simcoe. The DOC concentrations were found to be highest in Lake Simcoe, however, and this lake also had a unique relationship between DOC concentrations and conductivity, which suggested an additional and significant internal source of DOC in this lake. The temporal variations in the different optical characteristics of DOM in Lake Simcoe suggested that DOM qualitative transformations, be it through photooxidation or microbial degradation, are likely important processes in this lake. The variations in the quality and bioavailability of DOM have important implications for the cycling and biogeochemical processes of C in these systems. A more focused study on Lake Simcoe sources, transformation processes, and sinks of DOM would provide valuable information on the role of C on the DO cycle of this lake.

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