

Relationships between DOC concentration, molecular size and fluorescence properties of DOM in a stream

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Abstract

The patterns of dissolved organic matter (DOM) fluorescence properties were examined in a Precambrian shield stream over a seven-month field study. Unique spatial and temporal patterns of simultaneous changes were observed in dissolved organic carbon concentration (DOC), humic-like fluorescence intensity, maximum excitation and emission wavelengths and fluorescence index (the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm at an excitation wavelength of 370 nm). The spatial change indicates the alteration of DOM along the length of the stream, and temporal change corresponded to a drought event in August. In contrast to humic-like fluorescence, the protein-like fluorescence shows considerable variability, suggesting its ephemeral nature. There were strong relationships between humic-like fluorescence intensity, fluorescence index, maximum Ex/Em wavelengths, DOC concentration and molecular size of DOM. This study has significant implications to the understanding of the nature and biogeochemical cycling of DOM.

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1. Introduction

Dissolved organic matter (DOM) is an important component of the organic energy budget of natural aquatic ecosystems, and plays a significant role in affecting speciation and bioavailability of trace metals, solubility of organic pollutants, natural acidity and the attenuation of ultraviolet (UV) and visible radiation of waters (Schindler et al., 1996; Molot and Dillon, 1997; Moran et al., 2000). Therefore,

an understanding of the chemical and structural nature of DOM would be of great interest if one were to make predictions regarding the environmental effect of DOM in natural waters. For example, it has been demonstrated that DOM controls the watershed export of Hg deposited in precipitation (Mierle and Ingram, 1991), and that the complexation between DOM and trace metals, and between DOM and organic pollutants may be influenced by its molecular size (Whitehouse, 1985; Lin et al., 1995).

Unfortunately, due to its heterogeneous complexity and dynamic nature, DOM is especially difficult to characterize without fractionating it into smaller groups based on chemical or physical prop-

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erties. Therefore, the ability to predict the whole quality and structural nature of DOM from readily available measurements would be of great value. Dissolved organic carbon (DOC) concentration, fluorescence and molecular size (MS) are currently the most common parameters used to characterize DOM (Chin et al., 1994; Coble, 1996; Schindler et al., 1996; Molot and Dillon, 1997; Moran et al., 2000; McKnight et al., 2001), and relationships between them, if any, would be useful in predictive models. Evans et al. (1989) established a strong relationship between DOC concentration and MS of DOM in Ontario lakes, and found that the average MS can be predicted from DOC concentration and the ratio of catchment area to the surface area of a lake. Chin et al. (1994) demonstrated strong correlations between molar adsorptivity, total aromaticity and the average MS of all humic substances, and suggested that bulk spectroscopic properties can be used to quickly estimate the molecular size and their aromatic contents. McKnight et al. (2001) found that aromaticity and precursor sources were related to fluorescence index (the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm at an excitation wavelength of 370 nm).

The objective of this study was (1) to study the spatial and temporal patterns of changes in DOC concentration, fluorescence intensity, maximum fluorescence excitation and emission wavelengths and MS of DOM in a Precambrian shield stream, and (2) to examine their possible relationships. Environmental factors responsible for those changes and implications were also discussed.

2. Samples and methods

2.1. Study sites

Detailed information on study areas and sampling sites has been reported previously (Kothawala, 2002). Briefly, the study site is located in a 540 ha watershed on the Precambrian shield in South-central Ontario, Canada. The catchment studied, Hp 4 (Fig. 1), is one of the six watersheds of Harp Lake (45° 23'N, 79° 08'W). Hp 4, with a total area of 119 ha, is composed of minor till plain, thin till and exposed ponds. Water samples were collected at 15 sites along the length of the main tributary for examination of spatial patterns. Sampling site Hp 4-1 is located in a large wetland collecting headwaters from a primarily mixed deciduous and coniferous forest. An ephemeral stream, Hp 4-2, drains the large wetland and enters Swamp 2. The outflow of Swamp 2 is Hp 4-4. Hp 4-5 and Hp 4-7 are located further downstream in a section which passes through a primarily coniferous forest for 120 m, prior to reaching the *Sphagnum*-surrounded site Hp 4-9, Hp 4-10 is the first of three consecutive beaver ponds, representing study sites Hp 4-10, Hp 4-11 and Hp 4-12, respectively. Hp 4-13 is situated at the outflow of beaver pond Hp 4-12, while Hp 4-15, Hp 4-19, Hp 4-23 and Hp 4-27 are located at 40, 130, 250 and 370 m downstream of it. The final site was in the littoral zone of the lake, LK. These sample sites appear evenly spaced (Figs. 2–4) on the plots although they were not.

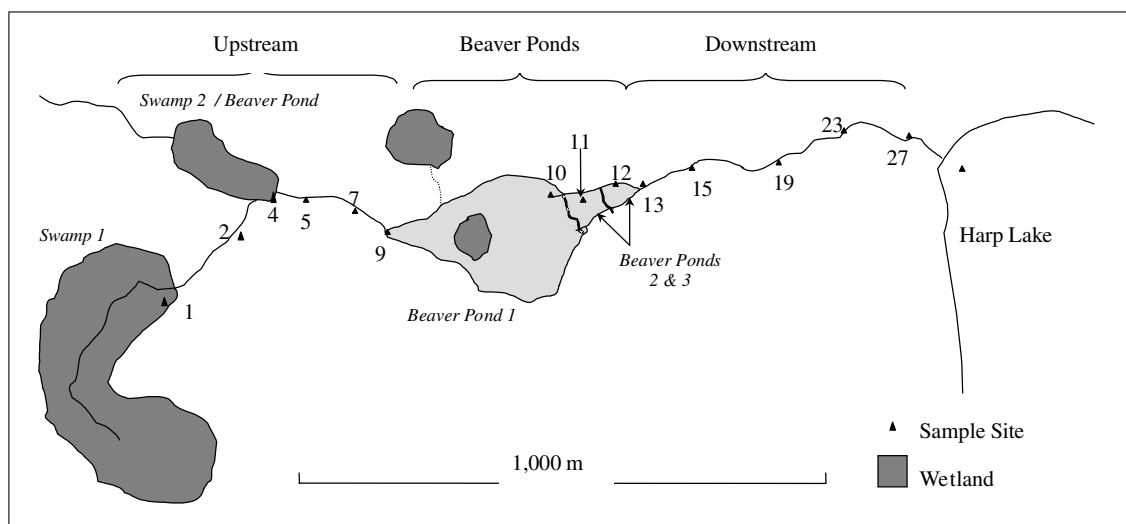


Fig. 1. Catchment Hp 4 and sampling sites.

Samples were collected from surface waters at each of the sites. They were filtered through 0.45 µm nylon syringe filters (Life Sciences, Ontario) before analysis.

2.2. Methods and materials

Total C (TC) and inorganic C (IC) were determined with a Shimadzu 5000 TOC analyser with potassium hydrogen phthalate as a standard. DOC was calculated from the difference between TC and IC.

For fluorescence measurement, a spectrofluorometer (SPEX, Jobin Yvon, Instruments S. A. Inc.) with a 150 W Xe short arc lamp and a multi-channel charge coupled device (CCD) detector was used. Excitation (Ex) wavelength was set from 250 to 550 nm, and emission (Em) wavelength from 250 to 700 nm, applying a bandwidth of 2 nm for both. A 0.25 mm slit width was chosen for both Ex and Em, and an integration time of 0.8 s was used. Each sample was scanned five times, and the resulting spectra were averaged. The fluorescence intensities were evaluated in quinine sulfate units

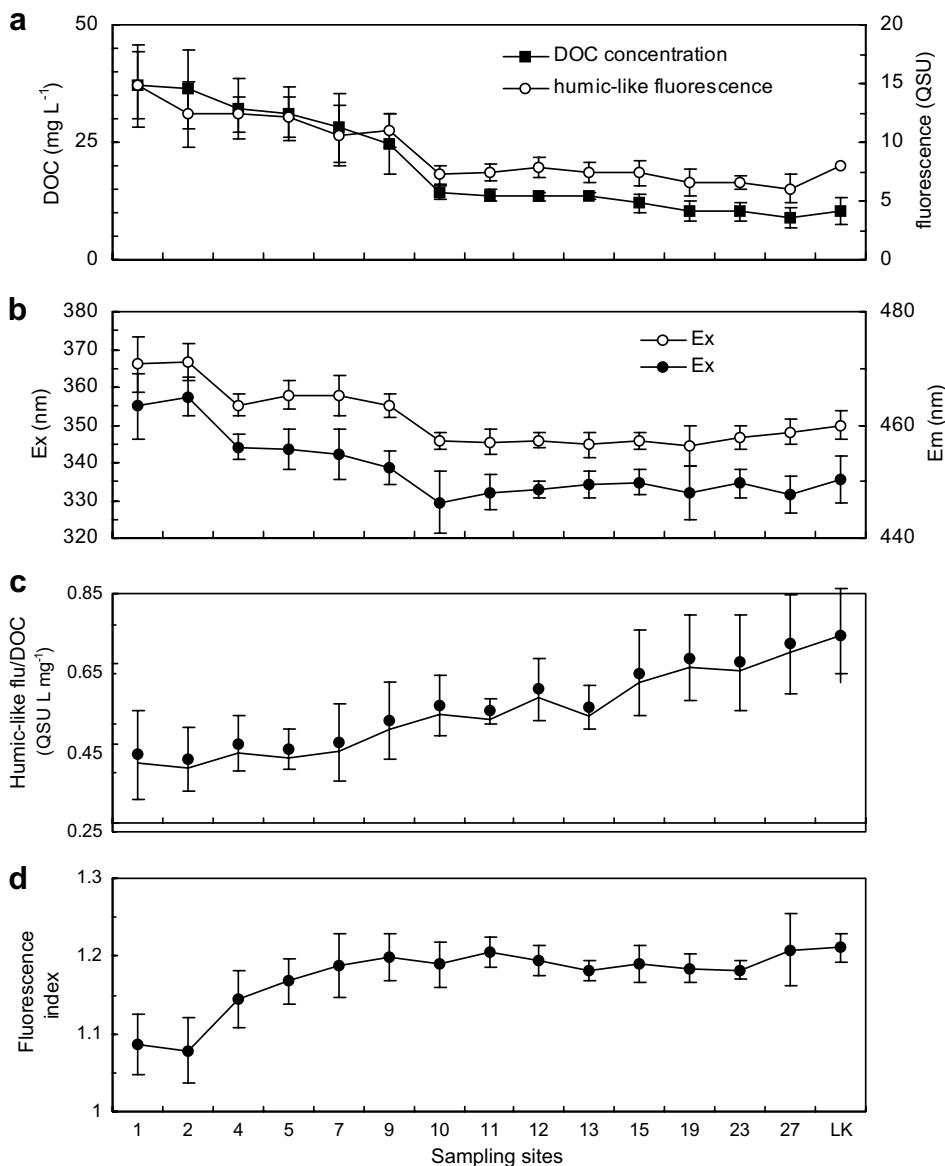


Fig. 2. Spatial changes in DOC concentration, humic-like fluorescence, fluorescence index, excitation and emission wavelengths of DOM, and ratio of humic-like fluorescence to DOC along the length of the stream.

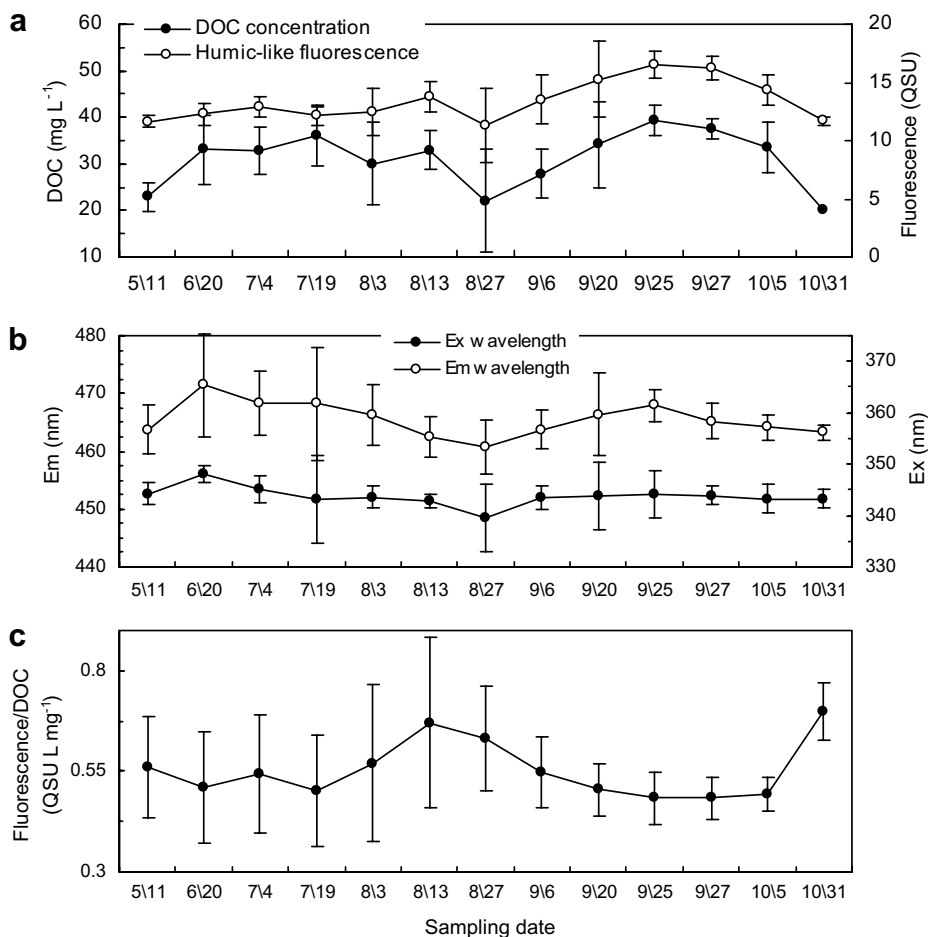


Fig. 3. Temporal changes in DOC concentration, humic-like fluorescence intensity, Ex and Em wavelengths of DOM, and ratio of humic-like fluorescence intensity to DOC in the stream.

(QSU); 1 QSU = 1 $\mu\text{g l}^{-1}$ of quinine sulfate monohydrate in a 0.05 M solution of H_2SO_4 at Ex/Em 350/450 nm after subtracting the blank fluorescence of Milli-Q water. The Ex/Em wavelengths and intensities of fluorescence maxima were used for characterization, and the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm at an excitation wavelength of 370 nm was calculated as a fluorescence index, as suggested by McKnight et al. (2001).

High-performance size exclusion chromatography (HPSEC) was used to estimate the MS of DOM (Kothawala, 2002). The HPLC system was equipped with a Waters 600 controller, a photodiode array detector and an autosampler. The flow rate was set at 0.5 ml min^{-1} . A Waters YMC-60 column (Waters Associates, Milford, USA) was used. The column was packed with a silica diol modified material with 5 μm gel bead diameter and 60 \AA pore

size. A 100 μl sample was injected onto the column, and was eluted with a phosphate mobile phase (0.1 M NaCl + 0.004 M phosphate, pH 6.8). MS calibration was based on polystyrene sulfonate standards (American Polymer Standards, USA), and in this study, the MS data were reported as number-average molecular weight, Mn (Kothawala, 2002).

3. Results and discussion

Fluorescence spectra of water samples in the stream generally exhibited two major fluorescence maxima: humic-like and protein-like fluorescence. The humic-like fluorescence peak occurred at Ex/Em 330–380/430–480 nm, and the protein-like fluorescence occurred at Ex/Em 298–305/332–335 nm. Fig. 2 shows spatial patterns of changes in DOC concentration and humic-like fluorescence characteristics of DOM for sites along the length of the

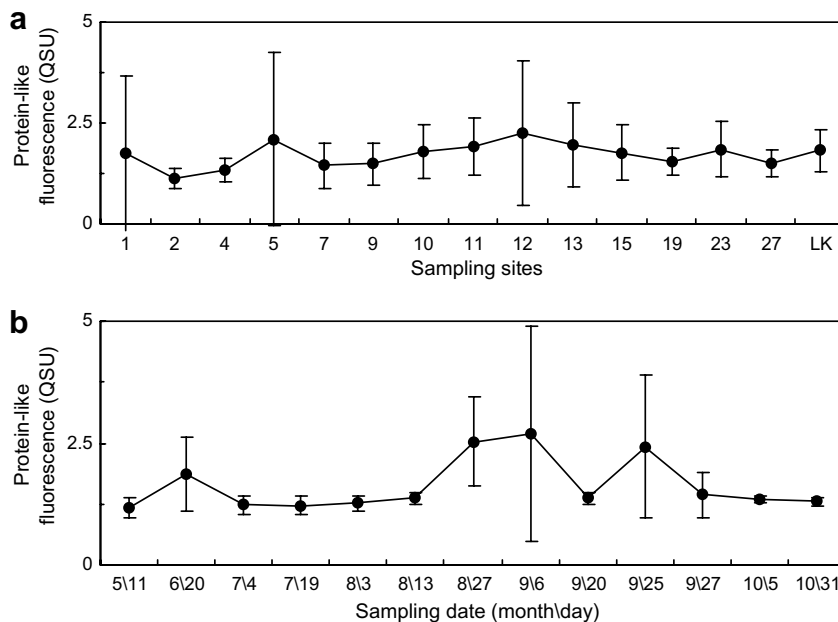


Fig. 4. Spatial and temporal changes in protein-like fluorescence intensity in the stream.

stream. All DOC concentration, humic-like fluorescence intensity and maximum Ex/Em wavelengths decreased, and fluorescence index increased along the length of the stream. Kothawala (2002) has demonstrated that dilution effects from groundwater are minor and suggests that other factors play an important role in these changes. The decrease in DOC concentration during transport has been previously found in other streams and rivers (Kaplan et al., 1980; Kaisa-Heikkinen, 1989). It has been reported that DOC, MS and fluorescence intensity decreased, and fluorescence maximum was shifted towards shorter wavelengths when DOM was under UV radiation (Melillo et al., 1983; Molot and Dillon, 1997; Moran et al., 2000). Maximum Ex wavelength of natural DOM was found to be red-shifted with increasing molecular size and apparent decrease in fluorescence efficiency (Hall and Lee, 1974; Wu and Tanoue, 2001a). The increasing fluorescence and DOC ratios (Fig. 2c) may provide further support that spatial MS distribution was shifted towards a lower distribution along the length of the stream (Kothawala, 2002). Hence, photo-oxidation, together with other processes including coagulation, sedimentation and selective adsorption of larger MS molecules to inorganic particles, metal oxides and bottom sediments (Stewart and Wetzel, 1981; Melillo et al., 1983; Wang et al., 1997), may contribute to the DOC decrease, and consequently

to changes in the fluorescence properties along the length of the stream.

The headwater sites, Hp 4–1 and Hp 4–2, were completely dry for the four-week period from August 3 to Sept 6; other sites had very low flow during this period (Kothawala, 2002). The major characteristics of DOC, humic-like fluorescence intensity and Ex/Em wavelengths corresponded to this drought event, as both DOC, humic-like fluorescence and maximum Ex/Em wavelengths were simultaneously lowered during the drought event (Fig. 3a and b). High DOC concentration has often been related to high discharge and low DOC concentration related to low discharge in many streams and rivers in the world (Manny and Wetzel, 1973; Meybeck, 1982; Kaisa-Heikkinen, 1989; Ivarsson and Jansson, 1994), and the results seem consistent with those previous reports. The simultaneous increasing DOC concentration, fluorescence intensity and Ex/Em wavelengths in the stream following the dry season may reflect the flushing of DOM stored in soils over the drought period. The increase in the ratios of fluorescence intensity to DOC in August (Fig. 3c) indicates that stream DOM is different through time. Ivarsson and Jansson (1994) reported that in a Northern Sweden stream during low flow season, older and more decomposed DOM from deep peat layers was drained during the dry season. Before and after the drought period,

comparatively large amounts of less decomposed DOM were washed out from the top soils and drained into the stream. This may hold true here as well. In addition, temperature was higher in the dry summer event, which may consequently enhance photodegradation and sedimentation of DOM, thus contributing to the increase in fluorescence efficiency.

Humic-like fluorescence and DOC concentration were strongly correlated (Table 1). McKnight et al. (2001) established a relationship between fluorescence index and the general source of dissolved fulvic acid in lakes, rivers and streams. Considering the predominantly humic-like fluorescence characteristics and low average fluorescence index (1.17 ± 0.05), it appears that the stream DOM was mainly allochthonous, and humic substances were the main component of DOM. Slight but consistent increases in fluorescence index (Fig. 2d), together with the occurrence of protein-like fluorescence (Fig. 4), indicate that autochthonous sources of DOM were added along the length of the stream. In a similar study of the Surumoni River in Venezuela, Battin (1998) suggested that the fluorescence

index, along with absorbance and DOC, may infer that the downriver reaches of the Surumoni River received autochthonous sources of DOM.

Protein-like fluorescence remained relatively constant both spatially and temporally, but occasionally increased in variability (Fig. 4). Larger variability and higher fluorescence intensities were found in summer time, Jun 20, Aug 28, Sept 6 and Sept 25 at sites Hp 4–1, Hp 4–5 and Hp 4–12, which are adjacent to the headwater swamps 1 and 2, and the Beaver ponds. Protein-like fluorescence was often associated with biological activities such as plankton blooms, phytoplankton and bacteria in aquatic environments (Traganza, 1969; Mopper and Schultz, 1993; Coble, 1996; Determann et al., 1996). The occasional increase, along with the invariable Ex/Em wavelengths of the protein-like fluorescence, may indicate specific heterotrophic activities in the swamp and beaver ponds in summer. The scattered spatial and temporal patterns of protein-like fluorescence, as compared to DOC concentration and humic-like fluorescence, imply considerable environmental variability, and indicate that this fluorescing DOM persisted for a period of

Table 1
Correlations between fluorescence properties, DOC and molecular size of DOM in the stream^a

y	X	Equations, and relation coefficient
Flu index	DOC	$y = -0.0023x + 1.22, R^2 = 0.30$
Flu	DOC	$y = 717.2x + 8376.6, R^2 = 0.87$
Ex	DOC	$y = 0.62x + 339.35, R^2 = 0.67$
Em	DOC	$y = 0.44x + 443.7, R^2 = 0.59$
Em	Ex	$y = 0.67x + 216.03, R^2 = 0.79$
Flu index	Em	$y = -0.0053x + 3.58, R^2 = 0.55$
Flu index	Ex	$y = -0.0031x + 2.26, R^2 = 0.30$
Flu index	Flu	$y = -0.0055x + 1.23, R^2 = 0.18$
M_n	Flu index	$y = -1228.3x + 2365.2, R^2 = 0.27$ (May–July) $y = 450x + 617.85, R^2 = 0.0098$ (August) $y = -2584.7x + 4360.7, R^2 = 0.27$ (Sept–Oct)
M_n	DOC	$y = 7.46x + 778.23, R^2 = 0.22$ (May–July) $y = 12.13x + 990.26, R^2 = 0.53$ (August) $y = 14.69x + 1028.1, R^2 = 0.83$ (Sept–Oct)
M_n	Flu	$y = 47.20x + 481.06, R^2 = 0.46$ (May–July) $y = 31.24x + 906.72, R^2 = 0.31$ (August) $y = 36.22x + 962.82, R^2 = 0.85$ (Sept–Oct)
M_n	Em	$y = 7.44x - 1699, R^2 = 0.26$ (May–July) $y = 12.95x - 3342.5, R^2 = 0.31$ (August) $y = 18.63x - 5209.6, R^2 = 0.82$ (Sept–Oct)
M_n	Ex	$y = 7.36x - 2400.1, R^2 = 0.22$ (May–July) $y = 20.24x - 7894.9, R^2 = 0.54$ (August) $y = 26.63x - 10697, R^2 = 0.74$ (Sept–Oct)

^a Units of DOC concentration, fluorescence intensity (Flu), Ex or Em wavelengths, number-averaged molecular size (M_n) are mg l^{-1} , QSU, nm and Da. All relationships are significant with $p < 0.01$ except for the correlation between M_n and fluorescence index in August.

time less than the water residence time in the stream. As the sampling sites were 10–100 m away from each other and sampling dates were several days apart, this persistence was likely on an order of hours or days. Similar short persistence of protein-like fluorescence has also been reported in two Maine estuaries (Mayer et al., 1999). The short life of protein-like fluorescence suggests that more detailed sampling and biological investigations are warranted. The protein-like fluorescence DOM fraction may be larger molecular size molecules (Wu and Tanoue, 2001a,b). In another study of the Harp Lake watershed, protein-like fluorescence fractions consisted of less than 10% of total DOM (Wu et al., 2003). Thus, it is possible that the transport of those large molecules was of little consequence to the fate of bulk DOM in the stream.

The decrease in DOC concentration along the length of the stream was accompanied by a series of simultaneous changes in DOM quality. The data further show that they were significantly correlated (Table 1). DOC concentration not only had a strong and positive correlation with fluorescence intensity, but also a significant and positive correlation with Ex and Em wavelengths (Table 1), with DOC concentration accounting for 89.7% of the variation in both fluorescence intensity, Ex and Em wavelengths. Ex wavelength was also significantly correlated with Em wavelength (Table 1). Shifts in Ex maxima are indicative of changes in absorption spectra and chemical composition, while shifts in Em maxima are indicative of changes in energy between the ground state and the first excited state. These could also have resulted from structural changes including the extent of the π -electron system and the number of functional groups (Senesi, 1990). Hence, the significant correlation between Ex and Em indicates that both the chemical composition and structure of DOM were systematically altered along the length of the stream. Both DOC concentration and humic-like fluorescence characteristics were well correlated with MS when the data were examined on a seasonal basis. The relationships of number-averaged molecular size (M_n) with DOC concentration, humic-like fluorescence intensity and Ex/Em wavelengths were significant and positive for samples collected from May–July and Sept–Oct, respectively (Table 1). During different seasons, various origin DOM and removal processes may contribute to the nature of DOM with varying proportions. The strength of the relationships in different seasons appears to reflect those changes. In a

previous study, Evans et al. (1989) established a strong correlation between M_n and DOC concentration for 20 Ontario lakes, with DOC concentration accounting for almost 70% of the variation in MS.

Statistical analyses (Table 1) also demonstrated that the fluorescence index had significant and negative relationships with DOC concentration, fluorescence intensity and Em and Ex wavelengths, with fluorescence index accounting for 58.2% of the variation in fluorescence properties (Ex and Em wavelengths and fluorescence intensity). Hence, the simultaneous patterns of change in both DOC concentration, Ex, Em and fluorescence intensity and fluorescence index strongly augment spatial and temporal change in DOM chemical and structural properties.

Because of the inherent chemical complexity of DOM, there are few simple chemical analyses to readily characterize DOM in meaningful ways in natural aquatic environments (McKnight et al., 2001). Evans et al. (1989) used DOC concentration and the ratio of catchment area to surface area of a lake to predict the MS of DOM in Ontario lakes. Chin et al. (1994) used molar absorptivity to predict the MS and aromaticity of humic substances. McKnight et al. (2001) found that the fluorescence index had a strong relationship with aromaticity, calculated from the ^{13}C NMR spectrum, for isolated fulvic acid samples. Strong relationships have found between DOC concentration, fluorescence intensity, fluorescence index, maximum Ex/Em wavelengths and MS in the stream. Therefore, it is possible to use a single DOC concentration or fluorescence measurement to predict physical and chemical properties of the whole DOM, i.e., MS and aromaticity, and even possibly precursor sources. The determination of the MS of DOM is a difficult problem, and results of various techniques are inconsistent; DOC analysis needs specific equipment and much time (Evans et al., 1989; Kothawala, 2002); aromaticity is usually determined by NMR spectroscopy and samples need preconcentration. Based on these established relationships, the easily available fluorescence measurement, which takes less than a second per analysis, would be one of the best parameters for the prediction of general DOM quality in the stream. However, this is only one study site and further studies need to be carried out before more general conclusions are reached.

These relationships can also be used potentially in combination with other water quality data to infer DOM characteristics relevant to biogeochemi-

cal modelling and monitoring of the stream ecosystem. It has been found that the metal-binding properties and bioavailability of DOM can vary significantly with MS, and MS has been found to be integrally related to chemical and fluorescence properties (Hall and Lee, 1974; Whitehouse, 1985; Lin et al., 1995; Wu and Tanoue, 2001a,b). In watershed ecosystems, as DOM is degraded, metal availability may change. As the physical and chemical nature of DOM changes during its transport from its source through the stream to the lake, its interaction with metals is altered. In modelling these changes and the response of streams to anthropogenic stresses and circumstances that may arise from climate change, parameters can be chosen on the basis of these relationships. Further, it has been demonstrated that fluorescence index can be used as an indicator of the source and aromaticity of dissolved fulvic acid in natural waters (Battin, 1998; McKnight et al., 2001). The difference in the fluorescence index may be accompanied by a shift in the maximum Ex/Em wavelengths, and an increase in fluorescence was correlated with a decrease in peak emission wavelength (McKnight et al., 2001). In this context, the relationship between fluorescence index, fluorescence intensity and maximum Ex/Em wavelengths, together with the relationship with DOC established here, provides basic data for the modelling and monitoring of DOM nature and biogeochemical cycling in the watershed ecosystem.

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