Source tracing of dissolved organic matter (DOM) in watersheds using UV and fluorescence spectroscopy

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Abstract

In aquatic ecosystems, dissolved organic matter (DOM) is an important source of detrital energy on which microorganisms rely. However, its dynamics are not well understood in an ecological context. By isolating specific watershed sources, the work reported in this thesis has attempted to characterize the seasonal patterns of DOM in the hyporheic zone of a temperate stream and to find the likely sources that contribute to this abundant pool of organic carbon. Hyporheic DOM characteristics described by UV spectroscopy indicated temporal rather than spatial dependence in relative allochthonous/autochthonous source in the fall. Excitation-emission matrices (EEMs) showed that hyporheic DOM was mainly comprised of fulvic- and humic-like fluorescence with small amounts of protein-like fluorescence. Cedar needles appeared to be an important and consistent source of this protein-like fluorescence in autumn. In contrast, DOM leached from birch litter was more dynamic in that its quality as an energy source decreased as the season progressed. Increases in dissolved organic carbon (DOC) concentrations from birch litter isolates were greater than those from cedar litter in early autumn, but less in late autumn. Although streambed biofilm was not significant in increasing DOC concentrations, it was also a source of protein-like fluorescence.
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Chapter 1: Thesis Introduction

Since this thesis draws from the fields of ecology and analytical chemistry, background information from both disciplines is presented. First, dissolved organic matter (DOM) will be defined and likely sources will be identified. The function of detrital energy in streams is discussed, followed by a short description of the breakdown process of leaf litter. The hyporheic zone as a unique biogeochemical environment that links surface with groundwater is explained. Finally, chemical measures of DOM using UV and fluorescence spectroscopy and their respective interpretations are introduced.

Definition and Properties of Dissolved Organic Matter (DOM)

Dissolved organic matter (DOM) contains dissolved organic carbon (DOC), which is present in all natural water bodies (Thomsen et al., 2002), is the world’s second largest carbon reservoir after fossil fuel deposits (Purves et al., 2001). Accordingly, its role in the global carbon cycle has generated much research interest, but is not well understood because of its heterogeneous structure.

DOM is defined as the fraction of natural water that can pass through a 0.45 µm filter (Allan, 1995) although the range used can be 0.22 – 1.2 µm (Aitkenhead-Petersen et al., 2003). It comprises proteins, peptides, amino acids, carbohydrates, and humic substances resulting from degradation of plant material by biological and natural chemical processes in terrestrial and aquatic environments (Hudson et al., 2007). Humic substances can be separated into 3 classes depending on their solubility at different pH: humins are not soluble under any pH conditions; humic acids are soluble if the pH is lower than 2; fulvic acids are soluble under all pH conditions (Hudson et al., 2007). Although the term DOM refers to all particles in solution, carbon is the quantitative parameter measured the most often (Findlay, 2006). Therefore, dissolved organic carbon (DOC) concentrations are usually used as a quantitative proxy for DOM (Findlay, 2006). DOC and DOM, in effect, are largely influenced by their sources and biogeochemical processes.
DOM Sources in a North-Temperate Stream

DOM inputs can be classified into two types: allochthonous (outside of the system) and autochthonous (within the system). In oceans, autochthonous inputs of DOM from phytoplankton are dominant (Stedmon and Markager, 2005), and DOM production in the mesopelagic and abyssal layers in the deep ocean exceeds that of riverine inputs (Yamashita and Tanoue, 2008). Allochthonous sources such as from plants and soils are important in coastal areas, but represent only 2-3% of oceanic DOM, overall (Opsahl and Benner, 1997).

However, in temperate streams, allochthonous sources of DOM are plentiful and may be more influential in determining DOM structure. Examples of these include: major input of groundwater that is relatively constant over time and highly refractory (i.e. biologically unavailable) (Allan, 1995); leaf litter input that is pulsed in autumn (Allan, 1995), and soil runoffs that contain processed soil material (Leichtfried, 2007). The above examples generally enter the stream either directly via aerial drift, or via advective transport of surface and ground waters (Aitkenhead-Peterson et al., 2003). Autochthonous sources of DOM include extracellular release from algae (mainly periphyton in lotic systems and phytoplankton in lentic systems) and from aquatic macrophytes that are of local importance and may show seasonal pulses due to an increase in photosynthetic activity in the summer months (Allan, 1995). But in temperate headwater streams where riparian vegetation can limit photosynthesis (Vannote et al., 1980), aquatic food webs require another energy source.

Detrital Energy in Food Webs

Aquatic food webs depend on large inputs of non-living organic matter as a detrital energy source, most notably leaf litter (Allan, 1995). This was first demonstrated in a temperate stream (Kaushik and Hynes, 1971), and has also been observed in lowland intermittent streams in southeastern Australia (Reid et al., 2008). However, in forested temperate streams, inputs of organic matter from within the stream may be more important in food webs than previously thought (Torres-Ruiz et al., 2007).

Microbes and fungi are essential to the decomposition of large amounts of leaf organic matter as they use it as a food source, and in turn, they become food for animals in higher trophic
levels such as invertebrates and fishes (Allan, 1995). In the case of invertebrates, many are omnivores that consume both detritus and bacteria (Hall and Meyer, 1998). This pathway, termed the microbial loop, describes how organic matter as energy is reintroduced into food webs via bacteria; it is still a relatively immature theory (Foreman and Covert, 2003) and varies depending on the ecosystem.

In pelagic systems, most of the carbon assimilated by bacteria is easily lost through the protozoan trophic levels (Hall and Meyer, 1998). But in streams, sediments play an important role in the microbial loop for several reasons. First, more bacteria can colonize sediment with larger surface area (Leichtfried, 2007) so that meiofauna (organisms that range from 40 µm to 500 µm in size [Palmer et al., 2006]), macroinvertebrates, and protozoans all are able to consume them (Hall and Meyer, 1998). Second, since sediments are in continual contact with surface waters, they provide an opportunity to hold DOM in place through abiotic sorption so that it can later be attacked by enzymes produced by the microbial community (Findlay, 2003). At the bottom of the food web, bacterial biomass and community structure depend highly on carbon source and quality (Hall and Meyer, 1998; Findlay, 2003). At any one point in time, there should be a series of bacterial species that are able to compete for the most bioavailable DOM components (Kaplan and Newbold, 2000); slower growing species will perhaps be more dominant in a community that is distant from the initial DOM source as they are better able to metabolize the recalcitrant components (Kaplan and Newbold, 2000). Leaf litter is an example of a DOM source that varies in its bioavailability as it degrades.

**Leaf Processing**

In temperate watersheds, autumn-shed leaves enter streams as a pulsed input and are processed in several stages (Allan, 1995). When leaves are soaked in water, they leach most of their soluble nutrients, organic and inorganic, in the first few days (Allan, 1995). However, trees resorb most of the nutrients from the leaves prior to abscission and the nutrients left are mostly cellulose and lignin, which are significant sources of energy that are unavailable to most animals (Benfield, 2006). The second stage is known as microbial conditioning, where microbes and fungi colonize the leaf’s surface and excrete enzymes to digest the leaf to soften its structure (Allan, 1995). In
the last stage, macroinvertebrates known as shredders continue to break up the dead leaves as a food source (Benfield, 2006).

There are physical, chemical, and biological factors that help control decomposition rates. Physically, deciduous leaves from birch or maple trees which have a softer structure will be broken down more easily by microbial activity (which is also influenced by water temperature [Gulis and Suberkropp, 2003]) and macroinvertebrates (Benfield, 2006), whereas leaves with higher lignin content such as those from coniferous trees are tougher and more resistant to degradation (Benfield, 2006). Chemically, processing rates of leaf litter are influenced by condensed tannin, N, C:N, and the lignin content of the leaves, which are species-dependent (Ostrofsky, 1997) and are closely related to the activities of macroinvertebrate predators and the rate at which shredders consume the litter (Hunter et al., 2003). Chemical defences of the leaf (Graca, 2001) and leaf quality, rather than stream differences (Leroy and Marks, 2006) are also relevant in determining processing rates. Biologically, decomposition rates of leaf litter in water are under the control of microorganisms as well as protists that consume these bacteria and act as a top-down control on bacterial growth rates to keep it in log phase (Ribblett et al., 2005).

Although leaf decomposition is a crucial and well-studied process in streams, less is known about a particular type of region underneath streambeds that is important in stream metabolism and integrates surface, groundwater and benthic processes.

**Hyporheic Zone**

The hyporheic zone beneath a streambed is a hydrological ecotone in which surface and subsurface waters mix due to streambed topography (Boulton et al., 1998) (Figure 1.1). The downwelling zone provides dissolved oxygen and organic matter to microorganisms and macroinvertebrates while the upwelling zone provides nutrients to areas downstream (Boulton et al., 1998; Kaplan and Newbold, 2000).

Understanding hyporheic zone function is important in stream ecology for several reasons: it provides a habitat for interstitial biota and nutrients for areas downstream (Sliva and Williams, 2005); it helps regulate stream metabolism and dissolved organic carbon dynamics by providing an area of solute uptake; and it is crucial in helping to develop and substantiate
hydrologic models (Dahm et al., 2006). Several studies have attempted to understand the role of hyporheic zones in heterotrophic metabolism and uptake in forest streams (Mulholland et al., 1997), as producers of dissolved organic carbon and carbon gases in temperate forested streams (Schindler and Krabbenhoft, 1998), and in changing dissolved inorganic nitrogen composition and concentrations in arid streams (Taleb et al., 2008). However, seasonal dynamics of this interstitial environment in temperate agriculturally impacted streams are poorly understood. By using UV and fluorescence spectroscopy, several measurements about DOM can be obtained in context to their bioavailability and quality as an energy source for microorganisms.

Measures of DOM Quality

Although DOC concentrations can be measured easily and accurately, alone they cannot describe DOM quality or bioavailability. Spectroscopy techniques such as UV and fluorescence are likely to be more informative in describing DOM character. The main advantage of these two techniques is that chromatographic fractionation and separation with resins (i.e., XAD) is not required. Separation techniques with resin can be slow and are not considered quantitative, so the DOM isolated using resin does not necessarily represent naturally-occurring DOM (Benner, 2003). Other advantages associated with UV and fluorescence spectroscopy are that they are quick and easy to use, require small sample sizes (~ 3 mL to fill a cuvette), and the associated instrumentation is commonplace and relatively inexpensive. This section of the introduction will introduce the theory of UV and fluorescence spectroscopy and the different indices used in this thesis to investigate aspects of DOM.

UV Spectroscopy

In general, bonding electrons of a chemical species are responsible for the absorption of UV-Vis radiation. Since the wavelengths of the absorption peaks are related to the types of bonds present, UV-Vis spectroscopy is useful for identifying functional groups in a molecule (Skoog et al., 1996). Essentially, all organic compounds are able to absorb electromagnetic radiation because their valence electrons are at a low energy level that can be excited to higher energy levels
(Skoog et al., 1996). The functional groups that are able to absorb UV-Vis radiation are termed chromophores (Skoog et al., 1996).

As DOM structure is heterogeneous (McKnight et al., 2003), it contains a variety of chromophores that are able to absorb electromagnetic radiation. Some examples are conjugated olfenic and aromatic structures with acidic, basic, and neutral functional groups (i.e., esters, ketones, phenols, alcoholic OH, amines/amides); these chromophores often overlap and absorb in the same region (Nollet, 2007). Hence, for natural water, this results in a UV-Vis spectrum that is broad and decreases with increasing wavelength (Fig. 1.2).

**Spectral Slope (S)**

Spectral slopes are calculated using a linear regression of ln (A(\(\lambda_0\))) versus wavelength range of 300 – 600 nm in the following equation (Markager and Vincent, 2000):

\[
A(\lambda) = A(\lambda_0) \exp[S(\lambda_0 - \lambda)]
\]

where A is the absorbance value at wavelength \(\lambda\) (nm), S is the spectral slope, and \(\lambda_0\) is the reference wavelength used (330 nm).

Although there is no standard method of calculating S values (researchers use various wavelength ranges, such as 300 – 700 nm [Helms et al., 2008], or 280 – 500 nm [Zhang et al., 2007]), S values typically range from 9.2 – 36.2 \(\mu\text{m}^{-1}\) depending on the system (Markager and Vincent, 2000). For example, S values are approximately 17 \(\mu\text{m}^{-1}\) in freshwater and about 14 \(\mu\text{m}^{-1}\) in the sea (Markager and Vincent, 2000). Higher S values indicate a steeper slope (Fig. 1.2), which indicates lower absorption at the longer wavelengths. Autochthonous sources of DOM do not absorb as strongly at these longer wavelengths because they are not as coloured, resulting in a steeper slope (Stedmon and Markager, 2001; Markager and Vincent, 2000).
Aromaticity

Specific UV absorbance (SUVA\textsubscript{280}) is calculated by dividing the absorbance values at 280 nm by DOC concentration (Mladenov et al., 2007; Chin et al., 1998). This value is expressed in units of \text{L.mg}^{-1}.\text{m}^{-1} and in general, if it is high (i.e., \geq 4 \text{L.mg}^{-1}.\text{m}^{-1}), the DOM will contain relatively higher amounts of compounds that are aromatic, macromolecular, and heterogeneous in structure (Nollet, 2007). Conversely, low SUVA values indicate DOM that is more homogeneous and other low-molecular weight compounds that are not as aromatic (Nollet, 2007). Although SUVA values can be measured using 254 nm, nitrates can also absorb at this wavelength (Mladenov, 2007). Then, measuring specific UV absorbance at 280 nm is perhaps a better alternative for characterizing aromatic carbon as the $\pi$-$\pi^*$ electron transition that is specific for compounds such as polycyclic aromatic hydrocarbons with two or more rings and anilines occurs between 270 and 280 nm (Nollet, 2007).

High aromaticity is reflective of a more terrestrial/macrophyte source and aged DOM, whereas low aromaticity is more associated with an algal source with non-aromatic and freshly created DOM (Moran and Covert, 2003). DOM that is more aromatic tends to be more recalcitrant to microbial degradation (McKnight et al., 2003) as microorganisms need the aid of extracellular enzymes or photoreactions to degrade them (Moran and Covert, 2003). Although allochthonous, aromatic DOM tends to decompose more slowly; its abundance in the ecosystem is thought to be important in providing a stable source of energy (Wetzel, 2003).

Average Molecular Weight

The E2/E3 ratio is calculated as:

$$E2/E3 = \frac{A_{254\text{nm}}}{A_{365\text{nm}}}$$

where \(A_{254\text{nm}}\) and \(A_{365\text{nm}}\) represents the absorbance at 254 nm and at 365 nm, respectively. The E2/E3 ratio is a proxy that is inversely related to average DOM molecular weight. The higher the ratio, the lower the molecular weight (Ågren et al., 2008; Dahlen, 1996). Although 254 nm is a measure of aromaticity, the E2/E3 ratio has been shown to increase with photolysis (Strome and Miller, 1978; Dalzell et al., 2009). Since photochemistry breaks down the larger components of
DOM, this would result in DOM that is simpler in structure and lower in molecular weight (Moran and Covert, 2003). Other examples of the E2/E3 ratio from the Georgia Bight ranged from 8–11 (unitless) (Helms et al., 2008), and range from 4–24 in Yongdam Reservoir, Korea (Hur et al., 2007).

The average molecular weight of DOM is an indicator of general reactivity and bioavailability (Sinsabaugh and Foreman, 2003). According to the size-reactivity model (Sinsabaugh and Foreman, 2003), molecules with a higher molecular weight are more recalcitrant (i.e., resistant to biological degradation) and more aliphatic in structure (molecules that are more aliphatic in structure are more simple and yield less energy when their bonds are broken). The size-reactivity model can be seen from either a trophic or a diagenetic perspective (Sinsabaugh and Foreman, 2003). From a trophic perspective (i.e., examining what is consumed), large molecules need to be degraded into smaller ones for uptake by organisms, which leads to the conclusion that smaller molecules have a shorter turnover time than larger molecules (Sinsabaugh and Foreman, 2003). From a diagenetic perspective (i.e., examining what is left), large molecules are continuously losing their reactive groups, resulting in smaller-sized refractory molecules (Sinsabaugh and Foreman, 2003).

Despite the different perspectives, the DOM observed in any particular ecosystem depends on whether it is dominated by aged or newly created carbon (Sinsabaugh and Foreman, 2003). Since freshwater systems are more eutrophic and dynamic compared to marine systems, diagenetic dynamics may be difficult to recognize (Sinsabaugh and Foreman, 2003). Therefore, for a headwater stream in which nutrients are not expected to be a limiting factor, low molecular weight compounds can be interpreted as better substrates than those with higher molecular weight (Tranvik and Jørgensen, 1995).

Fluorescence Spectroscopy

When a pair of electrons in the ground state absorbs energy, it is excited to a higher energy level (a singlet state). The electrons lose some of that energy in vibrational relaxation and return to the ground state (Fig. 1.3) (Skoog et al., 1996). As the electrons return to the ground state, light in the form of fluorescence is emitted (Skoog et al., 1996; Lackowicz, 2006). Due to vibrational
relaxation, the wavelength of light with which the electrons are excited is lower than the light that is emitted (also called the Stoke’s Shift) (Skoog et al., 1996).

Compared to UV, fluorescence is a more sensitive method (i.e., able to detect lower concentrations), but is not as suitable for determining quantities, as there is no reference material that is common to all types of natural water (Nollet, 2007). Since fluorescence measurements can be affected by factors such as pH, quenching, ionic strength, temperature, and redox (Skoog et al., 1996), the relative fluorescence measurements need to be standardized and corrected for inner filter effects and Raman scattering in order to compare between different instruments (Nollet, 2007).

**Fluorescence Index**

The fluorescence index (FI) is calculated by the equation:

\[
FI = \frac{\text{emission intensity at } 350 \text{ nm}}{\text{emission intensity at } 400 \text{ nm} \,(\text{at excitation wavelength of } 370 \text{ nm})}
\]

This equation was first derived by McKnight (2001), and indicates the source of fulvic acids in a given DOM sample. Fulvic acids, on average, comprise just over half of DOM in a typical river with a DOC concentration of 5 mg/L (McKnight et al., 2003). If the FI is ~1.4, this indicates an allochthonous source of DOM, whereas an FI of ~1.9 indicates an autochthonous source of DOM, specifically microbes (McKnight et al., 2001).

**Excitation-Emission Matrices (EEMs)**

EEMs were first developed by Coble (1996) to visualize the complex nature of DOM without time-consuming extraction methods. EEMs are generated by collecting emission spectra over a series of excitation wavelengths (see Figure 1.2). This creates a surface on which the main types of fluorescing compounds, or fluorophores, can be observed. EEMs are generally plotted as contour plots (a two-dimensional representation of three-dimensional data) and are read like topographical maps where more lines around a centre indicate an elevated area. On the x- and y-
axes, excitation wavelengths (in nm) and emission wavelengths (in nm) are plotted, respectively. Fluorescence intensities are presented by colour on the z-axis. The intensities are calibrated by dividing by the area of the Raman signal (excitation 348 nm emission 395 – 400 nm) to obtain Raman units (Baker, 2005).

In EEMs, DOM components will fluoresce in different locations on the surface. Therefore, the location of the fluorescing centre (i.e., a “mountain” on a contour map) indicates the presence or absence of the particular type of fluorescence. There are two locations for protein-like fluorescence: the first one is called “tyrosine” (excitation 275 nm, emission 305 nm) which often occurs together with the second, “tryptophan” (excitation 275 – 280 nm, emission 340 – 350 nm) (Stedmon and Markager, 2005; Mopper and Schultz, 1993). The tryptophan fluorescence also occurs at excitation 220 nm, emission 340 – 350 nm (Mopper and Schultz, 1993) but for simplicity, these fluorescing centres are labelled “P” (Fig. 1.2). These protein-like fluorescences are thought to originate from freshwater, planktonic bacteria (Elliott et al., 2006).

Fulvic-like fluorescence occurs at excitation 305 – 350 nm, emission 410 – 430 nm (Baker, 2001; Coble, 1996) while humic-like fluorescence occurs at excitation 230 – 260 nm, emission 420 – 450 nm (Coble, 1996). Although fulvic- and humic-acids are not indicative of any particular source, autochthonous sources of fulvic-acid are usually 10 – 30% of DOM in surface and groundwaters (McKnight et al., 2003). EEMs of two reference materials, Pony Lake Fulvic Acid and Suwannee River Natural Organic Matter, were obtained from the International Humic Substances Society (IHSS) and are shown in Figure 1.6. Their EEMs are similar in that they both show humic-like fluorescence, but fulvic-like fluorescence differs.

**Specific Thesis Objectives and Outline**

Although DOM is pertinent to ecologists and environmental chemists, the disparity of research approaches is evident. On the one hand, ecologists investigate the nutrient controls of food webs by examining responses from organisms at the community level or constructing carbon/nitrogen budgets. On the other hand, biogeochemists aim to develop and use new molecular tools to characterize DOM in order to better understand ecosystem function. The main motivation of the present research is to bridge the gap by using relevant analytical chemistry techniques to
examine the detrital energy (i.e., DOM pool) that will be available for microbial uptake over space and time.

Chapter 2 describes a study of the seasonal and spatial patterns of DOM in the hyporheic zone of the Speed River over a period of 12 months. Hyporheic water was sampled at depths of up to 1 metre using cores that were installed into the streambed (Fig. 1.3). Although seasonal DOC concentrations have been characterized previously for a temperate stream (Butturini and Sabater, 2000), this is the first time that UV and fluorescence spectroscopic techniques have been used. Some possible sources of the observed DOM are given and are a part of the results from the study described in Chapter 3.

Chapter 3 details a laboratory study of DOM leached from watershed sources on three separate occasions in the fall of 2007. Representing two allochthonous sources are leaf litter from white birch (Betula papyrifera) and white cedar (Thuja occidentalis); stream biofilm served as an autochthonous source. Based on vegetation characteristics of the study site, the abundance of these sources is believed to most likely influence DOM character at this time of year. A secondary goal of this study was to examine the role of microorganisms in the stream water in further degrading leaf litter and on biofilm.

In Chapter 4, a summary of the thesis and ideas for future research are presented.
Figures

**Figure 1.1.** A schematic of the hyporheic zone in which groundwater flow can enter from the stream bank sides and surface flow downwells, mixes with the groundwater, and upwells back to the surface again. Modified from C. M. Febria, unpublished.

**Figure 1.2.** Typical UV absorbance spectrum for natural water. The spectrum from 300 – 600 nm is log-transformed and modelled for spectral slopes.
Figure 1.3. A simplified Jablonski diagram indicating electrons that absorb light and move to a higher energy state will emit fluorescent light upon return to the ground state.
Figure 1.4. A schematic of the sampling core that was used to collect water samples underneath the streambed at various depths.
Figure 1.5. An example of an excitation-emission matrix (EEM) with the excitation wavelengths plotted on the y-axis and emission wavelengths on the x-axis. The locations of the protein-like fluorescence are labelled “P”, humic-like fluorescence labelled “H”, and fulvic-like fluorescence labelled “F”. Plotted with 20 contour lines, units on the z-axis are Raman units (R.U.).
Figure 1.6. Excitation-emission matrices (EEMs) of standards obtained from the International Humic Substances Society (IHSS): (a) Pony Lake Fulvic Acid and (b) Suwannee River Natural Organic Matter.
References


Chapter 2: Sources and seasonal patterns of dissolved organic matter (DOM) in the hyporheic zone

This chapter forms the basis of a manuscript submitted to *Hydrobiologia* as part of a special issue on the Great Lakes/St. Lawrence River Ecosystem (Conference: May 2008, Cornwall, Ontario).

Abstract

The hyporheic zone is a region underneath streambeds that integrates surface and groundwater. Although its location is central to biogeochemical linkages between the riparian zone, dissolved nutrients, and benthic biota, the seasonal quality and likely sources of dissolved organic matter (DOM) are not well understood. To investigate this, water from the surface and subsurface (at depths 20, 60, and 100 cm below the streambed) was sampled every 4 weeks from 2007 – 2008 in a third-order stream in southern Ontario. Using UV spectroscopy, measures of spectral slopes, aromaticity, and $A_{254}/A_{365}$ ratios were obtained. These characteristics reflected watershed processes such as shedding of leaf litter in the fall, and photochemical and biofilm influence in the spring and summer. The fluorescence index suggested that at the surface and in the downwelling zone, DOM microbial sources increased with depth in the sediment, regardless of the season. Excitation-emission matrices (EEMs) showed seasonally distinct, protein-like DOM components of bacterial origin that were stronger in the fall. Leachates from specific allochthonous DOM sources – leaf litter from *Betula papyrifera* (white birch) and *Thuja occidentalis* (white cedar) – and an autochthonous source, biofilm, were isolated and incubated with unfiltered surface water. EEMs from these leachates indicated that these sources could indeed help explain observed patterns of DOM in surface and subsurface waters. These results suggest that although DOM sources were relatively constant, biogeochemical processing within the hyporheic zone resulted a DOM pool that was temporally dynamic and altered the nature of organic matter transported downstream into lakes and rivers.
Introduction

Dissolved organic matter (DOM) is a heterogeneous, complex mixture present in all natural waters (Chin, 2003) and is the largest pool of carbon in aquatic ecosystems (Purves et al., 2001). DOM is becoming highly recognized for the role it plays in moderating the physical environment by: transferring carbon into the microbial food web (Findlay, 2003); complexing metals such as iron (Maranger and Pullin, 2003); transporting organic pollutants (Chin, 2003); and attenuating UV radiation (Clements et al., 2008).

Temperate regions experience dramatic seasonal changes in their flora and fauna, characterized by high primary productivity in the spring and summer months (April – August), high leaf litter production in autumn (September – November), and low productivity in winter (December – March). For small stream ecosystems, this represents seasonally and spatially different carbon sources that enter the watersheds. Will these large-scale processes be observable at the molecular scale? If so, then DOM source and character would be expected to be more allochthonous in the fall and more autochthonous in the spring and summer months.

However, this model of DOM source and character may be too simplistic after accounting for biogeochemical processes in the hyporheic zone that create a unique aquatic environment that resembles neither surface nor groundwater (Williams, 1993). Hyporheic zones are hydrological ecotones in which surface and groundwater mix (Dahm et al., 2006); they are supplied with oxygen and nutrients from upstream processes while they transform nutrients for downstream transport (Boulton et al., 1998). Although hyporheic zones are known to increase heterotrophic metabolism (Mulholland et al., 1997) and act as sources of dissolved organic carbon and carbon gases in forest streams (Schindler and Krabbenhoft, 1998), little is known about their seasonal DOM characteristics in north-temperate streams.

UV spectroscopic techniques have been used to characterize DOM using a variety of measures or proxies. Estimates of spectral slopes (S) can be used to determine the relative allochthonous or autochthonous DOM character since autochthonous sources absorb less strongly at longer wavelengths and will cause a steeper spectral slope compared to DOM with more allochthonous sources (Markager and Vincent, 2000). In short, higher S values correspond to higher autochthonous character of the DOM pool. Specific UV absorbance at 280 nm (SUVA$_{280}$) is positively correlated to DOM aromaticity (Chin et al., 1994; Chin et al., 1998). The
E2/E3 ratio, calculated as the absorbance at 254 nm divided by the absorbance at 365 nm, is negatively correlated to average molecular weight (Ågren et al., 2008; Dahlén et al., 1996).

Aromaticity and molecular weight are aspects of DOM that have important biological implications, particularly for microbial communities. Terrestrial DOM is mainly comprised of cellulose and lignin derivatives from which most aromatic compounds in aquatic environments originate (Wetzel, 2001). Higher aromaticity indicates a DOM pool that requires further breakdown in order to be assimilated, but is perhaps greater in energy than a DOM pool with relatively lower aromaticity (Fu et al., 2006; Weishaar et al., 2003). Similarly, DOM with a lower molecular weight is thought to be a better substrate for heterotrophic bacteria as it is assimilated more easily than more structurally complex, aromatic DOM (Tranvik and Jørgensen, 1995). However, their low concentrations and rapid cycling may cause bacteria to make use of the abundant, but humic and more aromatic portion of the DOM pool as an additional trophic resource (Kaplan and Newbold, 2003).

Using scanning fluorescence spectroscopy, emission spectra are gathered over a series of different excitation wavelengths that collectively, are referred to as excitation-emission matrices (EEMs). Developed by Coble (1996), EEMs give a complete picture of fluorescing DOM components such as: fulvic-, humic- like fluorescence (Coble, 1996; Baker, 2001), and protein-like fluorescence (Mopper and Schultz, 1993; Stedmon and Markager, 2005; Elliott et al., 2007). This technique has been used in a number of studies to characterize DOM from environments such as tidal marshes (Tzortziou et al., 2008), surface water in an upland peat catchment (Baker et al., 2008), and mangrove tannins (Maie et al., 2008).

Since streams and lakes are net heterotrophic, their metabolism is important to both atmospheric and terrestrial ecosystem processes (Williamson et al., 2008). Examining DOM sources and quality at the surface-subsurface interchange will improve our understanding of metabolic processes and the detrital energy resources available to microorganisms. The objectives of this study are to: 1) investigate seasonal patterns of DOM in the hyporheic zone and 2) find the most likely DOM sources by isolating known allochthonous and autochthonous material.
Materials and Methods

Study Site and Sampling

A third-order stream that enters the Speed River near Guelph, Ontario, Canada (43° 43’ N, 80°15’ W) was sampled every 4 weeks for surface and subsurface waters from September 2007 – November 2008 under baseflow conditions. Approximately 6 m wide, the stream is characterized by pool and riffle sequences and at the sampling site, the hyporheic zone is about 10 m in length (see Storey et al., 2003 for complete physical description and hydrological flow modelling). Due to streambed topography, surface waters can downwell into the subsurface in the downwelling zone (DW) that is located upstream of the hyporheic zone. Hyporheic water can also upwell and re-enter surface waters in the upwelling zone (UW) that is located downstream of the hyporheic zone. Both the downwelling and upwelling zones were sampled at depths of 20 cm, 60 cm, and 100 cm.

The land use in this area is retired agricultural, with small patches of mixed deciduous and coniferous trees, mainly white birch and white cedar respectively. Dissolved organic carbon (DOC) concentrations typically ranged from 3 – 12 mg/L and were usually highest in August, but experienced a peak in mid-fall (October). This is comparable to other streams in southern Ontario (Wilson and Xenopolous, 2008) and DOC concentrations generally decrease with increasing depth in stream sediments (Kaplan and Newbold, 2000).

Subsurface samples were obtained using piezometers installed into the streambed (Storey and Williams, 2004). Upon transport to the laboratory, all water samples were 0.22 µm filtered (cellulose nitrate, Millipore) on the same day and stored at -20°C in the dark.

On three separate occasions in fall 2007, allochthonous DOM sources, specifically leaf litter from white birch (*Betula papyrifera*) and eastern white cedar (*Thuja occidentalis*), and an autochthonous source, 12 cm² of biofilm from streambed rocks, were collected. These sources were incubated with unfiltered surface water collected at the same time for a period of 7 days.
**DOM Characterization**

Dissolved organic carbon (DOC) concentrations were measured as non-purgeable organic carbon using Shimadzu TOC-VCSH analyzer (Shimadzu Corp., Japan) using high temperature catalytic oxidation.

UV spectroscopy was done at room temperature using a Unicam UV Spectrometer (Unicam Instruments, UK), with samples scanned from 200 – 700 nm in a 1 cm quartz cuvette; Nanopure water scanned on the same day was used as a blank. Spectral slopes (S, \(\mu m^{-1}\)) were calculated (Markager and Vincent, 2000) for the wavelength range 300 – 600 nm. E2/E3 ratios were calculated (Dahlen, 1996) and specific UV absorbance at 280 nm (SUVA\(_{280}\)) was obtained by normalizing the absorbance at 280 nm to dissolved organic carbon concentration (Chin et al., 1994; Chin et al., 1998).

Fluorescence spectroscopy was done at room temperature using a PerkinElmer LS-50B (PerkinElmer Inc., UK) with a 1 cm quartz cuvette. Excitation-emission matrices (EEMs) were obtained by collecting 22 emission scans over the excitation wavelengths of 210 – 420 nm at 10 nm intervals. Both excitation and emission slit widths were 10 nm and a correction was made in excitation wavelengths as per the manufacturer’s instructions. There was no emission correction applied. UV-Vis absorbance data were used to correct for inner filter effects (IFE) (McKnight et al., 2001) and contour plots were generated using MATLAB (The Matworks, Natwick, MA). Fluorescence indices (FI) were also calculated according to McKnight (2001) to determine the relative DOM source (i.e. microbial vs. terrestrial). Two reference materials, Suwannee River NOM and Pony Lake Fulvic Acid, were obtained from the International Humic Substances Society and their FI were also measured to facilitate comparison of fluorometers from different manufacturers.

**Results**

Surface and subsurface samples were obtained monthly, however, in the winter months, subsurface waters could not be sampled because of frozen conditions. Surface water samples were obtained in the winter whenever possible.
**DOC Concentrations**

DOC concentrations in the downwelling zone generally increased except at 60 cm (Fig. 2.2a). In the upwelling zone, concentrations also increased in October 2007 before decreasing in the next month. Due to instrumentation troubleshooting, a DOC value could not be measured for surface water in February. In both the downwelling and upwelling zones, DOC concentrations decreased in May, but increased until late August, although in an inconsistent manner (Fig. 2.2a, b). This variation was especially evident at 60 cm in the upwelling zone (Fig. 2.2b). In the fall of 2008, DOC concentrations were similar to the year before in that they increased in October before decreasing again in November (except for 100 cm in the downwelling zone).

Spatially, DOC concentrations at 20 cm in both the downwelling and upwelling zones were slightly higher, or same as surface waters throughout the seasons. The deeper subsurface depths of 60 cm and 100 cm had lower DOC concentrations.

**UV Spectroscopy**

In fall 2007 (September – November), S values, or autochthonous character decreased sharply (in October) at the surface and 20 cm in the downwelling zone whereas they increased at 60 and 100 cm (Fig. 2.3a). At all depths in the upwelling zone, the autochthonous character increased during this period to levels above surface waters in October and November (Fig. 2.3b). During winter, S increased steadily throughout the winter in surface waters and this pattern continued throughout the summer at all depths except for 100 cm in the downwelling zone. At this location, the autochthonous character decreased before reaching a maximum in August. In fall 2008, S values decreased more dramatically than in the previous year, especially in the upwelling zone (Fig. 2.3b).

E2/E3 ratios were highly variable in surface, downwelling, and upwelling waters at the beginning of the fall (September) (Fig. 2.3 c, d). By October, the E2/E3 ratios increased slightly in the surface and downwelling waters except for 20 cm. In the upwelling waters, all depths had similar E2/E3 ratios, were lower than the surface, and increased in variability by November. In
contrast, waters at all downwelling depths in November showed similar E2/E3 ratios. Throughout winter, E2/E3 ratios at the surface varied widely from month to month and reached a minimum in January. But the ratio did not increase or decrease in any clear direction throughout spring or summer. During the transition from summer to fall, E2/E3 ratios generally decreased in surface and downwelling waters (Fig. 2.3c). In upwelling waters, E2/E3 ratios were closely mirrored at the surface and 20 cm in the upwelling zone, showed no clear pattern at 60 cm, and decreased at 100 cm (Fig. 2.3d).

SUVA\textsubscript{280} values decreased from September to October 2007 in downwelling waters, but this was more pronounced at 60 and 100 cm (Fig. 2.4a). In the upwelling zone, aromaticity generally increased at the surface, decreased in October at 20 cm, and generally decreased at 60 and 100 cm (Fig. 2.4b). In the spring and summer months, surface and downwelling waters increased and decreased in aromaticity with no clear direction (Fig. 2.4a). Maximum aromaticity was observed at: surface in July (Fig. 2.4a), 100 cm in the downwelling zone in June (Fig. 2.4a), and 100 cm in the upwelling zone (Fig. 2.4b). In the upwelling zone at 20 and 60 cm, aromaticity generally increased over the summer (Fig. 2.4b).

Fluorescence Spectroscopy

Fluorescence indices (FI) in fall 2007 decreased from September to October at all depths in the downwelling and upwelling zones except for 20 cm in the downwelling zone (Fig. 2.5c). The surface and downwelling values (Fig. 2.5c) mirrored the SUVA\textsubscript{280} values (Fig. 2.5a). Although FI increased in the downwelling zone in November, those in the upwelling zone decreased to a minimum (Fig. 2.5d). For surface waters, FI reached a maximum in November, fell steadily throughout the winter, then increased again throughout summer with a small peak in July. Deeper downwelling waters had consistently higher FI than shallower depths and surface waters throughout spring and summer except in July (Fig. 2.4c). Overall, surface waters and water at 20 cm in the downwelling zone were extremely similar during these months (Fig. 2.4c). FI in upwelling waters were not as predictable. In fall 2008, FI were not as low as those from the previous year, but reached a local minimum in October in the downwelling zone. However, FI were highest in the deepest subsurface and lowest in the surface – this was the same for both downwelling and upwelling waters (Fig. 2.4 c, d).
An excitation-emission matrix (EEM) of surface water from September 2007 was unavailable because of the large amounts of sample required to develop the fluorescence spectroscopy method. In Figure 2.5a, the following fluorescing centres are labelled as: humic-like fluorescence “H” (260 nm excitation and 380 – 460 nm emission); fulvic-like fluorescence “F” (320 – 340 nm excitation and 410 – 430 nm emission); and protein-like fluorescence “P” (220 nm and 275 – 280 nm excitation and 340 – 350 nm emission). At 20 cm in both the downwelling and upwelling zones (Fig. A-1a and Fig. A-2a, respectively), some protein-like fluorescence was observed in addition to the fulvic- and humic-like fluorescences. The EEMs from the other depths were similar to each other. In October 2007, some protein-like fluorescence could also be observed at the surface (Fig. A-1d) and at all depths in the upwelling zone (Fig. A-2d-f). The November EEMs showed protein-like fluorescence at the surface and 100 cm in the downwelling zone (Fig. 2.5 a, g), and humic-like fluorescence in all locations.

Only surface samples were obtained in January and February 2008 due to frozen conditions. In January, stronger fulvic-like fluorescence was observed compared to February but in both samples, humic-like fluorescence was observed (Fig. A-3a, b). In April, samples from upwelling depths of 60 cm and 100 cm were unavailable, again due to frozen conditions. Some protein-like fluorescence was observed at 100 cm in the downwelling zone in April (Fig. A-4d), but at the shallower depths and at the surface, only fulvic- and humic-like fluorescence was observed (Fig. A-4a-c). In May, the EEMs from the surface (Fig. A-4e) and the downwelling zone (Fig. A-4f-h) were similar in their fulvic- and humic-like fluorescence. At 20 cm in the downwelling zone and 60 cm in the upwelling zone, protein-like fluorescences were observed (Fig. A-4f, A-5c), while small amounts were observed at 60 and 100 cm in the downwelling zone (Fig. A-4g, h) and at 100 cm in the upwelling zone (Fig. A-5d).

In June 2008, humic- and fulvic-like fluorescences were observed at the surface, downwelling, and upwelling zones (Fig. 2.5, 2.6). The EEMs from July and August 2008 were very similar at the surface and subsurface in their fulvic- and humic-like fluorescences (Fig. A-6, A-7). Some protein-like fluorescence was observed at 20 cm in the downwelling zone in July (Fig. A-6b); small amounts were seen at 20 and 60 cm of the downwelling zone (Fig. A-6c, d) and at all depths in the upwelling zone (Fig. A-7a – c). In August, some protein-like fluorescence was observed at 20 cm in the downwelling zone (Fig. A-6f) while strong fulvic-like fluorescence was observed at 20 cm and 60 cm in the upwelling zone (Fig. A-7d, e).
In September and October 2008, the EEMs indicated mostly humic-like fluorescence at the surface, downwelling and upwelling zones (Fig. A-8, A-9). The EEMs from 2008 were not as comparable to the ones from the previous year, perhaps due to the fact that samples in 2008 were collected about 10 days earlier than the samples in 2007. The September samples at the surface and at 20 cm and 60 cm in the downwelling zone were stronger in fulvic-like fluorescence (Fig. A-8a, A-9a, b).

The EEMs from November 2008 again were not as comparable to the ones from the previous year, although small amounts of protein-like fluorescence could be observed at 100 cm in the downwelling zone (Fig. A-10d).

EEMs of isolated Speed River watershed DOM sources are shown in Figure 2.7. Birch leachates had strong fulvic- and humic-like fluorescence, and small amounts of protein-like fluorescence (Fig. 2.7a). This EEM appeared similar to the ones obtained from the surface, downwelling and upwelling zones in the spring (Fig. A-4, A-5) and in June (Fig. 2.6b). Protein-like fluorescence was strong in cedar leachates in addition to the fulvic- and humic-like fluorescence (Fig. 2.7b). The EEM of biofilm leachate did not have any protein-like fluorescence, and looked similar to the EEM at 20 cm in the downwelling zone (Fig. 2.7c).

Discussion

Fall 2007

From September to November 2007, S values, SUVA$\text{\textsubscript{280}}$, and FI decreased in October in the downwelling zone. These parameters pointed to a DOM pool that had increased in allochthonous character, had decreased in aromaticity, and had a more terrestrial source – likely due to the pulsed leaf litter in October, also reflected by the increase in DOC concentrations. Although aromaticity was low, nutrients such as carbohydrates and proteins that leached most quickly from leaf litter (Allan, 1995) do not have highly aromatic structures. E2/E3 ratios were variable in October, but were essentially the same in November, which was in contrast to what was observed in the upwelling zone – ratios were congruent in October, but became more variable in November, especially at 100 cm. In the upwelling zone, S generally increased, SUVA$\text{\textsubscript{280}}$
decreased slightly in October, and FI fell to minimums in November. The processes that resulted in a DOM pool that increased in autochthonous character and decreased in aromaticity were not immediately obvious. However, in both downwelling and upwelling zones, the FI minimums occurred simultaneously as increased variability in E2/E3 ratios, or molecular weight. This implied that terrestrial sources contributed compounds of different size classes to the DOM pool first in the downwelling zone in October and then, in the upwelling zone in November.

Although excitation-emission matrices (EEMs) showed mainly fulvic- and humic-like fluorescences in September and October 2007, the protein-like fluorescence in the upwelling zone in October suggested a possible decomposition of cedar litter in the hyporheic zone (see following interpretation of sources). EEMs in November showed greater spatial variation in downwelling waters than in upwelling. Protein-like fluorescence can be attributed to a bacterial origin (Elliott et al., 2006), likely enzymes produced by a particular microbial community to break down leaf litter (Suberkropp and Klug, 1976; Benfield, 2006). This was not seen in the shallower depths possibly due its generally low concentrations (Kaplan and Newbold, 2003).

Winter and Early Spring 2008

From January to April 2008, increasing S values and decreasing FI in surface waters were likely results of longer periods of sunlight and spring snowmelt that would have brought more soil runoff into surface waters. Although FI indicated allochthonous sources, photochemical reactions would have broken the recalcitrant substances into more labile ones (Moran and Covert, 2003) thereby increasing S. Unfortunately, we were not able to obtain a DOC value in February and cannot be certain of the aromaticity. But our results are in general agreement with other studies in that more aliphatic (i.e., simpler) structures increased during snowmelt (Ågren et al., 2008). Again, molecular weights did not show any obvious pattern during this period.

The EEMs from surface waters in January and February supported this idea as the fulvic- and humic-like fluorescences were evident, suggesting low primary productivity during these months and the only sources of DOM being from the surrounding soils (Ågren et al., 2008). However, in April, the protein-like fluorescence observed at 100 cm in the downwelling zone
could be a result of algal-sourced DOM that was not assimilated in the deep subsurface (see below).

*Summer 2008*

From May to August 2008, DOM was very dynamic at both the surface and in the deep subsurface. In May, 100 cm in the downwelling zone, aromaticity was low, S was high, and molecular weight was extremely low. This is likely due to the bloom of filamentous algae that is most visible on Speed River riffles in May; fresh, algal DOM is labile, low in aromaticity and molecular weight (Moran and Covert, 2003). But how was this DOM in surface waters transferred to the deep subsurface, apparently bypassing the shallower downwelling waters? A possible scenario is that the labile DOM was transferred to the downwelling zone, but was quickly taken up by the biofilms present on streambed rocks and in the shallow stream sediments (i.e., 20 and 60 cm). This was likely not the case as microbial activity is generally lower in the colder temperatures of the deep subsurface (Kaplan and Newbold, 2000), which might have resulted in labile DOM that was not assimilated. The EEMs supported this idea because protein-like fluorescence was observed at 20 cm in the downwelling zone and 60 cm in the upwelling zone, indicating their existence at these depths, but perhaps they were not in large enough concentrations to affect the UV spectroscopic measurements (fluorescence is more sensitive than UV [Skoog, 1996]). Furthermore, labile, algal DOM cycles quickly (i.e., minutes) (Wetzel, 2003) and this likely explains the somewhat inconsistent measurements from both techniques, as it took an average of 30 minutes to sample surface and groundwater in the field under good weather conditions.

In June, the most notable change in DOM characteristics occurred at 100 cm in the downwelling zone. DOM was aromatic, more allochthonous in character, higher in molecular weight compared to May, and was more terrestrial in source than at any other point in the season – suggesting some influence from the surface and shallow subsurface that are more terrestrial in their sources. From the EEMs, small amounts of protein-like fluorescence were observed which, like the previous month, suggested algal-derived DOM that was unused.
In July, S reached a maximum at 100 cm in the upwelling zone, was high in aromaticity, had relatively low molecular weight, and was microbial in source. These DOM characteristics were also like those observed at the surface. Although DOM with high aromaticity was not expected from microbial exudates and other autochthonous sources (Moran and Covert, 2003), the increased aromaticity at the surface could have resulted from photoreactions that rendered labile DOM into components that are less bioavailable (Moran and Covert, 2003). However, in the upwelling zone, a different process was likely; the aromaticity could have resulted from mobilization of particulate organic matter that was buried in the hyporheic zone (Trulleyova et al., 2003) and had already undergone extensive biogeochemical processing by benthic biota. The increased aromaticity was not seen at the other depths; perhaps they were assimilated by microorganisms in the shallow subsurface.

The protein-like fluorescence observed at 20 cm in the downwelling zone was also observed in relatively smaller amounts in the upwelling zone, suggesting a microbial assimilation of this DOM component at the shallower depths. In contrast, protein-like fluorescence increased slightly from the downwelling to upwelling zone at 60 and 100 cm, implying a production in the deep subsurface of this DOM in the hyporheic zone, likely from microbial sources attached to stream sediments.

In August, at 100 cm in the downwelling zone, the peak in S values was not accompanied by extreme values of aromaticity, molecular weight, or FI. This suggested that while DOM may have had a highly autochthonous character, this parameter alone could not be used to predict the ecological consequences of the DOM pool. The FI was highest at 20 cm in the upwelling zone and this correlated with strong fulvic-like fluorescence in the EEM. Since these characteristics were not observed in the downwelling zone, this implied microbial production of this DOM within the shallow subsurface.

Fall 2008

Differences in the regional climate in 2007 and 2008 were possible reasons for the observed annual variability in the parameters; 2007 was dry while 2008 was wet. DOC concentrations were somewhat conserved between the two years; they generally increased from early to late
summer and into October. The high concentrations at the end of the summer (August) could be attributed to biological senescence at the end of the production season while concentrations generally increased in October, likely due to leaf litter (Wilson and Xenopolous, 2008).

Annual variation was also observable for S; it was more temporally and spatially congruent in October 2008 than in the previous year. For example, in 2007, the deeper subsurface (60 and 100 cm) in downwelling waters did not decrease until November, and in 2008, the shallow subsurface at 20 cm experienced the seasonal low in September. S values in the upwelling zone in 2007 did not decrease, but did so dramatically the following year. This suggests that although leaf litter inputs occurred mainly in October, small amounts of litter entering the stream earlier in September or later in November also led to a more allochthonous DOM pool in these months. However, FI values showed more consistency between years, although they did not decrease as dramatically in 2008 as they did in 2007. In September, the EEMs indicated strong fulvic-like fluorescence in surface and 20 cm in the upwelling zone. Since the FI at these locations were low, it was likely that this fulvic-like fluorescence were of a terrestrial, rather than microbial source, possibly due to runoff (Allan and Castillo, 2007) or advective transport of allochthonous DOM (Aitkenhead-Petersen et al., 2003). Although EEMs can give an idea of how strong the fulvic-like fluorescence is, FI are valuable because they can distinguish between terrestrial and microbial sources (i.e., McKnight et al., 2001). In general, the EEMs were not consistent between 2007 and 2008; this could be due to the earlier sampling date (by about 10 days) in 2008. Since autochthonous, labile DOM cycles quickly (Wetzel, 2003), it is likely that protein-like fluorescence exists in low concentrations and is sensitive to sampling date.

Spatial Trends and Sources

DOC concentrations showed spatial trends in that the surface and shallow subsurface (20 cm) were higher than the deep subsurface (60 and 100 cm). This agreed with the general thought that DOC concentrations decreased with increasing depth into the subsurface (Kaplan and Newbold, 2000). This observation could perhaps be explained by the fact that surface and shallow subsurface would receive inputs from the surrounding soils (Allan and Castillo, 2007), whereas in the deeper subsurface, the water that seeps through the deeper soil horizons was not as rich in
organic carbon (Allan and Castillo, 2007). In general, the DOC concentrations in the upwelling zone were more variable than in the downwelling zone, which likely resulted from hyporheic processing that have yet to be understood.

FI showed strong spatial trends throughout the year, suggesting that microbial, autochthonous sources contribute relatively less to DOC concentrations than allochthonous sources. In both the downwelling and upwelling zones, the deep subsurface was stronger in microbial source than the shallow subsurface and surface waters. Despite seasonally different carbon inputs to headwater streams (Pusch et al., 1998) and different hyporheic flow (Fraser and Williams, 1998; Storey et al., 2003), it appeared that DOM source was determined spatially. This conformed to expectations in that DOM was mainly influenced by microbial inputs in the deeper subsurface (i.e., 100 cm) and at the surface and shallow subsurface, by terrestrial DOM sources such as advective transport from the surrounding soils (Aikenhead-Petersen et al., 2003) and runoff (Allan and Castillo, 1995). This trend was more consistent in the downwelling zone than in the upwelling zone, which suggested that additional DOM processing occurred within the hyporheic zone that changed the nature of the DOM that was transported downstream.

In general, average molecular weight showed no clear pattern temporally or spatially. This can be attributed to the heterogeneity of DOM and that there are present at any one time reactive and labile portions in each class size of molecules (Sinsabaugh and Foreman, 2003). This is in contrast to the study by Trulleyova and co-workers (2003) who found that molecular weight was generally higher in the spring and summer than in fall and winter.

EEMs of birch litter leachates revealed that they closely resembled the DOM observed in the summer. Leachates from cedar litter showed that it was most likely responsible for the protein-like fluorescence observed in the hyporheic DOM, and biofilm was a contributor of fulvic- and humic-like fluorescence. Since this assay was done in late November 2007, the leaf litter on the ground would have undergone more drying and structural breakdown than the leaves that entered the stream directly (Bärlocher, 1992). Nevertheless, leaf litter is a crucial source of protein- and fulvic-like fluorescence and its gradual breakdown by macroinvertebrates over the course of the year may have resulted in the stronger fulvic-like fluorescence observed in the hyporheic EEMs in spring and summer.
Critical Assessment of UV/Fluorescence Indicators Used

Since there is no single spectral method that can unambiguously describe DOM content (Nollet, 2007), multiple parameters were used. The degrees to which these proxies could illustrate DOM characteristics varied from successful (fluorescence index) to vague (E2/E3 ratios).

In general, spectral slopes were relatively successful at discerning the allochthonous/autochthonous character of DOM over the seasons and showed some consistency in between the years. Although SUVA$_{280}$ did not indicate any obvious pattern, this could be explained by the lack of consistency in the DOC concentrations in 2007 and 2008. The fluorescence index was arguably the most successful of all the indicators; even though some differences between the two years were observed, the observed values were all expected. EEMs were only able to provide qualitative information, but did give some insight as to the type of DOM that was observed. Their use could be further extended by statistically modelling their contributing fluorescing components using parallel factor analysis (Stedmon and Bro, 2008). But this method of modelling requires many sample sizes and EEMs that were collected at a higher resolution (i.e., excitation wavelength increments of less than 10 nm) than what was employed here.

However, the lack of any spatial and temporal pattern in the E2/E3 ratios raises the question as to whether this indicator is not useful as a proxy of molecular weight, or whether this reflected actual molecular weight variations in the DOM. Further investigation will need to be conducted to distinguish between these two possibilities. The varying success of the parameters used in this study stress the need to use multiple indicators in order to characterize DOM; it is unlikely that they will provide useful insight when used alone.

Conclusion

In this study, DOM from the hyporheic zone was characterized and compared to isolated leachates from specific watershed DOM sources. Watershed processes such as leaf litter input in the fall resulted in changes in DOM aromaticity, allochthonous character, and average molecular weight. During spring and summer, DOM at the surface and deep subsurface were the most
dynamic; although highly aromatic, the DOM was autochthonous in character and microbial in source. At the surface, this indicated photochemical reactions that decreased the bioavailability of labile, algal-derived DOM and at the subsurface, mobilization of buried particular organic matter. DOM source did not reflect temporal trends in the watershed, but indicated spatial trends – relative microbial source increased with depth into the subsurface, especially in the downwelling zone. Comparisons of the EEMs of hyporheic zone DOM and isolated leachates suggested that birch and cedar leaf litter contributed to fulvic- and protein-like fluorescence. Protein-like fluorescence observed in the spring and summer was likely from biofilm and microbes attached to stream sediments. Although the spectral parameters used were not always successful at discerning a clear spatial or temporal pattern on their own, when they were used together, they were able to provide some insight into the nature of DOM in the hyporheic zone. Overall, our results demonstrate that while hyporheic zones are predictable in DOM source, they are areas of active biogeochemical processing, which result in a temporally dynamic DOM pool that will ultimately affect downstream reaches.
Figures

Figure 2.1. Map of the study site near Guelph, Ontario, Canada. Reproduced with permission from the Grand River Conservation Authority, the smaller map shows the study area with the watershed area outlined.

Figure 2.2. DOC concentrations in the (a) downwelling zone and (b) upwelling zone from September 2007 – November 2008.
Figure 2.3. Seasonal trends of S, spectral slopes in the (a) surface and downwelling zone and (b) surface and upwelling zone. E2/E3 ratios over the same time period in (c) surface and downwelling zone and (d) surface and upwelling zone. Values for surface waters are the same in (a) and (b), as well as (c) and (d); numbers in the legend indicate depth below streambed in centimetres.
Figure 2.4. Seasonal trends of SUVA_{280} in the (a) surface and downwelling zone and (b) surface and upwelling zone. Fluorescence indices (FI) over the same time period in (c) surface and downwelling zone and (d) surface and upwelling zone. Values for surface waters are the same in (a) and (b), as well as (c) and (d); numbers in the legend indicate depth below streambed in centimeters.
Figure 2.5. Excitation-emission matrices (EEMs) of surface and downwelling waters in (a – d) November 2007 and (e – h) June 2008 demonstrate that the spatial variation in fall versus spatial congruence in the summer. As an example, the EEM in (a), fulvic-like fluorescence is marked with “F”, humic-like fluorescence with “H”, protein-like fluorescence with “P”. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure 2.6. EEMs of upwelling waters in (a – c) November 2007 and (e – f) June 2008 show the relative lack of spatial variation in upwelling waters in both fall and summer compared to those in the downwelling zone. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure 2.7. EEMs from isolated Speed River watershed DOM sources, (a) birch, (b) cedar, and (c) biofilm, after 24 hours of incubation in the presence of microorganisms with surface water collected in late November 2007. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
References


Chapter 3: Characterizing dissolved organic matter (DOM) isolated from specific allochthonous and autochthonous sources in a temperate stream

Abstract
Every autumn, in temperate regions, leaf litter enters headwater streams as a pulsed source of detrital energy that guides bottom-up structuring of aquatic food webs. However, it is unknown exactly how this leaf litter, microorganisms in the water column, and other dissolved organic matter (DOM) produced within the stream will affect DOM characteristics and dissolved organic carbon (DOC) concentrations. To investigate this, DOM was leached from two allochthonous sources: white birch (*Betula papyrifera*) and white cedar (*Thuja occidentalis*); and one autochthonous source, streambed biofilm, for a period of 7 days on 3 separate occasions in the fall of 2007. In one set of the incubations, microorganisms from the water column were filtered out to determine their effects on concentration and character. Deciduous leaf litter was responsible for high, short-term increases in DOC concentrations whereas the amounts leached from conifer needles were relatively constant each month. Using UV spectroscopy, changes to DOM characteristics such as aromaticity, spectral slopes, and molecular weight were mainly determined by source and implied microbial assimilations of first the labile DOM pool, and then the aromatic, recalcitrant pool. Excitation-emission matrices (EEMs) collected using fluorescence spectroscopy suggested that cedar litter was the most important source of protein-like fluorescence and that the nature of the fluorescing DOM components changed in the presence of microorganisms. The results of this study demonstrate the need to examine both DOC concentrations as well as DOM quality in order to understand the heterotrophic energy sources that maintain and fuel lotic food webs.

Introduction
Dissolved organic matter (DOM) is perhaps one of the most important components of the global carbon cycle. It is mainly comprised of dissolved organic carbon (DOC) (Findlay, 2006) and is
the most abundant pool of carbon in aquatic environments (Fisher and Likens, 1973; Battin et al., 2008). Among other functions, DOM is also a consistent resource for heterotrophic bacteria (Williamson et al., 2008), controls biogeochemical processes such as iron and phosphate chelation (Maranger and Pullin, 2003), and essentially links terrestrial and aquatic ecosystems because the carbon flow from plants and soils to DOM is significant in the terrestrial carbon budget (Jaffé et al., 2008).

The latter is especially true for streams in temperate regions. Among allochthonous DOM sources such as soil runoff and groundwater (Allan and Castillo, 2007), leaf litter is most abundant in the fall and is presumably responsible for the observed increase in DOC concentrations (Meyer et al., 1998; Wilson and Xenopoulos, 2008b). This annual input of heterotrophic energy is relatively large-scale and remains a resource for microorganisms and benthic invertebrates throughout the following winter, spring, and summer until the leaves are shed again the next fall (Kaushik and Hynes, 1971). Leaf decomposition is a well-known process in which leaves enter the stream by wind action or directly from bankside vegetation, have their soluble nutrients leached from them, followed by microbial colonization of their surfaces, and finally, physical abrasion by shredders (Allan and Castillo, 2007). Coniferous needles (from white cedar in this study), have higher lignin content and decompose slower than deciduous leaves (from white birch), as their tougher cuticles are more resistant to microbial colonization (Bärlocher et al., 1978). Although it is known that leaf litter enhances mercury methylation in aquatic ecosystems (Tsui et al., 2008) and that its quality affects utilization rates by aquatic decomposers (Tuchman et al., 2002), the role it plays in shaping DOM character has yet to be determined.

In contrast to leaf litter, autochthonous sources such as releases from primary production, microbial and cellular exudates, and viral lysis (Bertilsson and Jones, 2003) are generated throughout the year. In streams, biofilms that coat the surfaces of rocks, sediment, and decaying wood are complex aggregates of bacteria, algae, and exopolymeric matrices that contain high microbial biomass and are responsible for much of the primary production (Fischer, 2003; Lock et al., 1984). Biofilms respond to different DOM sources (Olapade and Leff, 2006) and change the food web structure at the higher trophic levels by preferentially assimilating labile carbon (Wilcox et al., 2005). DOM characteristics from biofilm also have yet to be determined.
Since DOM is not subject to the same settling forces as particulate organic matter, it can travel over much longer distances (Battin et al., 2008). Consequently, its structure is altered by spatially and temporally varying sources, biotic and abiotic processes (McKnight et al., 2003), and characteristics of the surrounding terrestrial ecosystem (Wilson and Xenopoulos, 2008). DOM structure may be complex, but UV and fluorescence spectroscopy are fast and simple methods to characterize DOM and its components. Using UV spectroscopy, several proxies that describe the DOM pool can be obtained: aromaticity, indicated by specific UV absorbance at 280 nm, or SUVA$_{280}$ (Chin et al., 1998; Chin et al., 1994); average molecular weight (indicated by E2/E3 ratios, or absorbance at 254 nm divided by absorbance at 365 nm; (Dahlen et al., 1996); and relative allochthonous character, indicated by spectral slopes, or S values, (Markager and Vincent, 2000). Increasing aromaticity and molecular weight generally indicate an increase in allochthonous sources (Ågren et al., 2008; Dahlen et al., 1996) while decreases in S values indicate a more allochthonous character (Markager and Vincent, 2000). Using fluorescence spectroscopy, fulvic-, humic-, and protein-like fluorescence components can be detected by obtaining emission spectra over a series of excitation wavelengths, collectively called excitation-emission matrices (EEMs) (Coble, 1996).

Examining these chemical parameters gives insight into the resources that are available to microorganisms. Aromatic DOM tends to be older and allochthonous in source (Moran and Covert, 2003). Its need to be broken down by extracellular enzymes before microbial assimilation (Arnosti, 2003) likely explains its slower degradation rates and accumulation in aquatic ecosystems – however, this provides an important ecosystem function as it acts as a stable source of energy (Wetzel, 2003). Microorganisms usually favour DOM with a lower average molecular weight as this is easily assimilated even though it provides less energy than DOM with a larger molecular weight (Kaplan and Newbold, 2003).

In incubation treatments with leaf litter, biofilm, and presence/absence of microorganisms from the water column, we examined modifications to DOC concentrations and DOM characteristics in order to address two main hypotheses. First, birch litter is a more important DOC source at the beginning of autumn because it is degraded relatively faster than cedar litter; biofilm is not expected to contribute to DOC concentrations. Second, DOM source is an important predictor of DOM quality and composition, but biogeochemical processing by
microorganisms in the water column would result in differences observed in aromaticity, spectral slopes, molecular weight, and fluorescing components.

Materials and Methods

Study Site and Sampling

Allochthonous and autochthonous sources of organic matter were collected every 4 weeks in late September, late October, and late November in and adjacent to a third-order stream that enters the Speed River near Guelph, Ontario (43° 44’ N, 80°15’ W) (Fig. 3.1). The stream measures 6 m wide and contains riffle-pool sequences along its length. Leaf litter from white birch (*Betula papyrifera*) and eastern white cedar (*Thuja occidentalis*) were used as allochthonous sources as these represent the major vegetation types around the stream. For the birch leaf litter, the leaves were randomly selected from the ground. For the cedar litter, brown leaves still attached to the branches were picked at random. Potentially, there may have been differences in the microorganisms present on the leaf litter, but litter traps were not used in order not to interfere with a simultaneous, natural field study at the same location. As an autochthonous DOM source, 12 cm² of biofilm was scraped from randomly selected rocks on the streambed. 20 L of surface water from the stream were collected at the same time.

Experimental Design

After each month’s collection, the leaf litter was allowed to air dry for 24 hours, and the surface water was 0.22 μm filtered (cellulose nitrate, Millipore, USA) in order to physically remove the microorganisms. The leaf litter was not autoclaved in order to mimic the natural process as closely as possible; some colonization and decomposition by terrestrial hyphomycetes was likely. The design of the experiment is illustrated in Figure 3.2. Each incubation (in autoclaved 2 L glass jars) with 2 replicates and contained 1 L of 0.22 μm, filtered stream water (900 mL for the incubations to which microorganisms would be added), and a source of organic matter (either 10 g of birch, 10 g of cedar litter, or 12 cm² of biofilm). No organic matter was added to the control.
All the incubations were allowed to acclimatize for 24 hours and were maintained in a 4 °C cold room. 100 mL aliquots of unfiltered stream water were added as a treatment while the other treatment did not contain microorganisms from the water column. 250 mL water samples were collected from each of the incubations for dissolved organic carbon (DOC), UV, and fluorescence spectroscopy analyses and 0.22 µm filtered (GF/F filtering for DOC analysis) at day 1 and day 7. The samples were stored in HDPE scintillation vials at -20 °C. The incubations were then covered with No.1 Whatman filters.

Chemical Analyses

Dissolved organic carbon (DOC) concentrations were measured as non-purgeable organic carbon using a Shimadzu TOC-VCSH analyzer (Shimadzu Corp., Kyoto, Japan) employing high temperature catalytic oxidation. UV spectroscopy was performed using a Unicam UV Spectrometer (Unicam Instruments, UK), with samples scanned from 200 – 700 nm in a 1 cm quartz cuvette at room temperature; Nanopure water scanned on the same day was used as a blank. Spectral slopes (S, µm⁻¹) were calculated (Markager and Vincent, 2000) for the wavelength range 300 – 600 nm. E2/E3 ratios (absorbance at 250 nm divided by absorbance at 365 nm) were calculated (Dahlen et al., 1996) and specific UV absorbance at 280 nm (SUVA₂₈₀) was obtained by normalizing the absorbance at 280 nm to dissolved organic carbon concentration (Mladenov, 2007; Chin et al., 1998). The measurements mentioned above were also collected simultaneously from the control incubations and used as a baseline for the incubations with an added DOM source.

Fluorescence spectroscopy was done at room temperature using a PerkinElmer LS-50B (PerkinElmer Inc., UK) with a 1 cm quartz cuvette. Excitation-emission matrices (EEMs) were obtained by collecting 22 emission scans over the excitation wavelengths of 210 – 420 nm at 10 nm intervals. Excitation and emission slit widths were 10 nm and a correction was made in excitation wavelengths as per the manufacturer’s instructions. There was no emission correction applied. UV-Vis absorbance data were used to correct for inner filter effects (IFE) (McKnight et al., 2001) and contour plots were generated using MATLAB (Matworks, Natwick, MA).
Statistical Analyses

Average-linkage hierarchal analysis and Spearman Rank correlation coefficients were generated using SPSS v. 13 (SPSS Inc. Chicago, IL).

Results

Birch litter was responsible for the largest DOC increases in September and October while cedar needles leached relatively the same amount of organic carbon from one month to the next (Fig. 3.3 a – c). By late autumn, the amount leached from birch was less than cedar (Fig. 3.3a). Birch incubations with a microbial inoculation generally experienced a higher increase in DOC concentrations, but statistically, they were not significantly different from the incubations with microorganisms (Fig. 3.3a).

UV Spectroscopy

SUVA\textsubscript{280} values, or aromaticity, increased in all incubations in September except for biofilm (Fig. 3.3d). In both birch and cedar incubations containing microorganisms, the increase in aromaticity was not as marked as those incubations without microorganisms (Fig. 3.3d). In the next month, aromaticity decreased in the birch, cedar, and biofilm incubations containing microorganisms (Fig. 3.3e). In contrast, incubations without microorganisms increased in aromaticity only in the birch incubations (Fig. 3.3e). In November, aromaticity changes were almost zero in the birch incubations while they decreased in the cedar incubations and the biofilm incubation without microorganisms (Fig. 3.3f). In contrast, aromaticity increased when biofilm was incubated with microorganisms (Fig. 3.3f).

In September, S values, or autochthonous character, decreased in the cedar and biofilm incubations and in the birch incubation without microorganisms (Fig. 3.4a). For the birch and biofilm incubations in October, S values decreased in those containing microorganisms and increased in those without microorganisms (Fig. 3.4b). For the cedar incubations, changes in S
were minimal (Fig. 3.4b). By November, S values were highly variable in all incubations, particularly cedar and biofilm (Fig. 3.4c).

Changes to E2/E3 ratios were minimal in September (Fig. 3.4d). In the next month, E2/E3 ratios increased in all the incubations without a microbial inoculum while they decreased for the incubations with microorganisms (Fig. 3.4e). However, the decrease was minimal in the cedar incubation (Fig. 3.4e). In November, the E2/E3 ratios were again variable (Fig. 3.4f).

The average-linkage dendrogram generated using DOC concentrations and proxies measured with UV spectroscopy showed that DOM character was mainly determined by source (Fig. 3.5). Spearman rank correlation coefficients (Table 3.1) indicated that: changes in SUVA$_{280}$ were negatively correlated with changes in E2/E3 ratios; changes in S were negatively correlated with changes in SUVA$_{280}$ and positively correlated with changes in E2/E3 ratios. Although other correlations are reported, only the ones mentioned above were statistically significant.

**Excitation-Emission Matrices (EEMs) from Birch Litter Incubations**

In Fig. 3.6a, the various locations of the fluorescing components are labelled: protein-like (excitation 220 nm and 275 nm, emission 340 – 350 nm, labelled “P”; Coble, 1996; Mopper and Schultz, 1993); fulvic-like (excitation 320 – 340 nm, emission 410 – 430 nm, labelled “F”; Coble, 1996); and humic-like (excitation 230 – 260 nm, emission 420 – 450 nm, labelled “H”; Coble, 1996). The birch incubations in September indicated fulvic-like fluorescence in the incubation with microorganisms (Fig. A-11a, b). Microorganisms appeared to increased protein-like fluorescence as more was observed after the incubation period (Fig. A-11b).

In October, EEMs of incubations at Day 1 with microorganisms showed protein-like fluorescence (Fig. 3.6a) that was also present in smaller amounts in the incubation without microorganisms (Fig. 3.6c). The protein-like fluorescence observed at the lower excitation wavelength in the incubation with microorganisms was not observed in the incubation without microorganisms (Fig. 3.6a – b). Compared to the incubation with microorganisms, fulvic-like fluorescence was stronger in the incubation without microorganisms (Fig. 3.6b). This is noted by the difference in scale, as represented by the colour. Humic-like fluorescence was observed only
in the incubation with microorganisms (Fig. 3.6a). After 7 days, fulvic-like fluorescence was observed in the incubation with microorganisms (Fig. 3.6b) while small amounts of protein- and humic-like fluorescence were observed in the incubation without microorganisms (Fig. 3.6d).

In November, the EEMs at Day 1 indicated humic- and fulvic-like fluorescence and some protein-like fluorescence (Fig. A-12a, c). However, the nature of the fluorescence changed after the 7 days in the incubations containing microorganisms (Fig. A-12a, b); changes were minimal in the incubation without microorganisms (Fig. A-12c, d). Part of these results were presented in Chapter 2, but have been included here to facilitate comparison.

**EEMs from Cedar Litter Incubations**

Cedar incubations in September indicated stronger protein-like fluorescence compared to the birch litter (Fig. A-11e-h). Also, the fulvic-like fluorescence was not as strong at the beginning of the incubation period as after in both types of incubations. Interestingly, the fulvic-like fluorescence in the incubation without microorganisms (Fig. A-11h) was higher than in the one containing microorganisms (Fig. A-11f).

In October, EEMs indicated the presence of fulvic-, humic-, and protein-like fluorescence although it was stronger in the incubation without microorganisms (Fig. 3.6e, f). After 7 days, fluorescence of the various DOM components increased in both the incubations with and without microorganisms (Fig. 3.6g, h), as indicated by the scale in the colour bars.

The protein-like fluorescence from the cedar litter incubations in November increased in fluorescence after the incubation period regardless of the presence of microorganisms (Fig. A-12 e – h). The fulvic-like fluorescence after the incubation period was also interesting in that it appeared microorganisms had changed the fulvic-like fluorescence from two fluorescing centres (i.e., “spots”) to one (Fig. A-12f, h).
EEMs from Biofilm Incubations

The DOM leached from biofilm in September indicated some protein-like fluorescence (Fig. A-13a), but this decreased after the incubation period (Fig. A-13b). In the absence of microorganisms, the fulvic-like fluorescence changed after the incubation period (Fig. A-13d). In October, EEMs showed fulvic- and humic-like fluorescence (Fig. A-13d), although the fulvic-like fluorescence was minimal in the incubation with microorganisms at day 1 (Fig. 3.7a). Fulvic-like fluorescence increased slightly after the incubation period in the presence of microorganisms (Fig. 3.7b). In contrast, in the incubation without microorganisms, the small amount of protein-like fluorescence was not observed after 7 days (Fig. 3.7d). The November EEMs did not indicate strong protein- nor fulvic-like fluorescence (Fig. A-14a, c). Fulvic-like fluorescence changed in the incubation containing microorganisms after the incubation period (Fig. A-14b).

EEMs from Incubations with Only Surface Water

In September, EEMs of the incubations with only surface water showed protein-like fluorescence that was stronger in the incubation with microorganisms (Fig. A-13e) than the incubation without microorganisms (Fig. A-13g). However, after the incubation period this decreased along with fulvic-like fluorescence in both types of incubations (Fig. A-13f, h). In the next month, EEMs of surface water with a microbial inoculum showed humic- and fulvic-like fluorescence (Fig. 3.7e). This was also observed in the incubation without microorganisms (Fig. 3.7g). After the incubation period, the nature of the fulvic- and humic-like fluorescence changed in both types of incubations (Fig. 3.7f, h). In November, microorganisms in the water column did not appear to affect fluorescence; the EEMs indicated humic-like fluorescence (Fig. A-14e, g) that did not change after the incubation period (Fig. A-14f, h).
Discussion

Implications for Field Observations in Fall 2007

Seasonal field observations (S values, E2/E3 ratios, and SUVA$_{280}$) for surface waters were presented in Chapter 2. For this discussion, only the incubations containing microorganisms will be discussed as these mimicked the natural environment more closely. S values in surface waters decreased from September to October before increasing again in November (Chapter 2). The results from this manipulation study did not entirely explain field observations because the DOM sources tested mostly decreased the S values (Fig. 3.4a – c). This was also the case for E2/E3 ratios that increased from September to October and decreased in November for surface waters; E2/E3 ratios from birch, cedar, and biofilm incubations neither increased nor decreased in a clear direction. In the field, surface waters increased in SUVA$_{280}$ from September to November and although the DOM sources increased in their aromaticity in September, they generally decreased or had no effect in the other months.

These DOM sources were chosen because of their relatively higher abundances and likelihood of determining DOM quality, which was supported by results from the dendrogram. However, the inability of the lab study to explain the field observations suggested that naturally-occurring DOM is more complex and likely an amalgamation of more biogeochemical processes than we were able to test.

Birch Litter as a DOM Source

In September, the amount of DOC leached from birch litter was the highest compared to the other DOM sources. However, the presence of microorganisms did not significantly increase the amount of DOC leached – perhaps a result of decomposition by hyphomycetes of the leaf litter prior to its entrance into the stream (Bärlocher, 1978).

DOM leached from birch leaf litter in the absence of microorganisms indicated an increase in aromaticity, decrease in autochthonous character, and increase in molecular weight (decrease in E2/E3 ratio). These parameters conformed to expectations because they suggested that leaf litter DOM was indeed allochthonous in character. However, when this DOM was
incubated with an inoculum of microorganisms from surface waters, aromaticity did not increase as much, and instead S increased, and molecular weight decreased. This result was not surprising, as microorganisms could have taken up the aromatic portions of the DOM, leaving behind a DOM pool that was more autochthonous, lower in molecular weight, and lower in aromaticity. From the EEMs, fulvic-like fluorescence was leached with the aid of microorganisms from the water column; this was supported by the comparably low fulvic-like fluorescence in the incubation without microorganisms (Fig. A-11c). Microorganisms increased protein-like fluorescence as more was observed after the incubation period (Fig. A-11b). Overall, this indicated that microorganisms were important in mobilizing DOM from the surface of the leaf litter in the first 24 hours (Allan and Castillo, 2007).

The increases in DOC concentrations from the birch leaf litter incubation treatments were again the highest in October, and the highest of all the incubations in any month. This supports field observations that fall DOC concentrations experience an annual increase in streams of this region (Wilson and Xenopolous, 2008a). In the incubation without microorganisms, aromaticity increased, indicating an increase in allochthonous-sourced DOM. But the increase in S and decrease in molecular weight indicated the opposite – perhaps the result of low-molecular weight nutrients such as proteins and carbohydrates leached from he leaf litter (Allan and Castillo, 2007).

This apparent contradiction could perhaps be explained by change observed in the fulvic-like fluorescence of the EEMs. Although fluorescence decreased overall, the nature of the fluorescence appeared to have changed, possibly increasing aromaticity. Microorganisms in the water column decreased aromaticity and autochthonous character and increased molecular weight; these parameters together indicated that the microorganisms used the labile, lower molecular weight portions of the DOM. The decrease in aromaticity could be explained by the decrease in protein- and humic-like fluorescence in the EEMs.

In November, birch leaf litter did not increase DOC concentrations regardless of whether there was a microbial inoculation or not, likely indicating significant decomposition by hyphomycetes before entering the stream. Net changes in aromaticity were minimal in both incubations, implying that there was more than one process occurring with opposing effects on aromaticity. The EEMs indicated stronger humic- and fulvic-like fluorescence compared to the
previous months, which decreased after the incubation period. This lack of protein-like fluorescence was perhaps a consequence of the increased decomposition of birch litter prior to entrance into the stream and perhaps, an increasingly poor source of detrital energy.

Without microorganisms, the DOM leached from birch litter decreased in autochthonous character and increased in molecular weight, which was expected from an allochthonous-sourced DOM. In the incubations with microorganisms, the allochthonous character also increased and the molecular weight decreased. This decrease pointed to the microbial use of the more recalcitrant, aromatic portions of the DOM, but since the allochthonous character increased, that conclusion cannot be supported. However, this was perhaps an indication that multiple biogeochemical processes occurred at similar magnitudes. The EEMs from these incubations were similar to the ones incubated with microorganisms, but lower in fluorescence. This implied that microorganisms in the water column became more important in degrading leaf litter in late autumn.

_Cedar Litter as a DOM Source_

In the incubations in September, the DOC increased in both incubation treatments were within the margin of error of each other. This suggested that the microorganisms in the water column played an insignificant role in degrading cedar needles. This was also evident in the EEMs; the stronger protein-like fluorescence at the beginning of the incubation period in the treatment containing cedar litter compared to birch litter implied that microorganisms were not crucial in mobilizing this DOM component from the litter.

In incubations without microorganisms, the DOM increased in aromaticity and molecular weight and decreased in autochthonous character. This was expected, as the needles were an allochthonous source. The effect of microorganisms on cedar litter was similar to that found in the birch DOM – microorganisms decreased aromaticity, increased autochthonous character, and decreased molecular weight. Here, a microbial uptake of the aromatic portions of DOM seems plausible, leaving behind a DOM pool that was less aromatic, more autochthonous, and lower in molecular weight. In the EEMs, the fulvic-like fluorescence in the incubation without microorganisms was higher than in the one containing microorganisms, implying microbial
assimilation of this fluorescing component. Interestingly, the fulvic-like fluorescence was not as strong at the beginning of the incubation period as after in both types of incubations, so this increase was more temporally dynamic.

In the October incubation treatments containing only cedar litter, aromaticity increased slightly, but their autochthonous character increased and molecular weight decreased. Although the last two parameters suggested a breakdown of the more aromatic portions of DOM into more autochthonous and lower molecular weight components, the increase in aromaticity did not support this idea. However, since this increase was essentially zero, it was possible that the larger DOM components broke into smaller parts without changing its aromaticity. Observations from the EEMs indicated that the fluorescence increased, but changes to structural complexity of the humic-like fluorescence (as indicated by the decrease in contour lines; see Fig. 3.6e, f) suggested that this DOM component and perhaps, aromaticity, degraded in the presence of microorganisms.

The effect of microorganisms in these incubation treatments decreased DOC concentrations and aromaticity. The decreased autochthonous character and increased molecular weight suggested a microbial uptake of the labile and smaller DOM components. The decreased aromaticity seemed to contradict this, but a possible explanation is that the aromatic portion of the DOM pool was broken down into the more labile components by the microorganisms as an additional resource. This was also supported by these EEMs as the humic-like fluorescence (and aromaticity) decreased and fulvic-like fluorescence increased slightly. In the EEMs of both types of incubations, the appearance of protein-like fluorescence at the beginning and end of the incubation period implied that the microorganisms in the water column did not play an important role in mobilizing this DOM component from the needle surface and that these resources were leached gradually.

In November, the DOC concentration increased in the incubations with cedar litter, was the highest compared to the other DOM sources, and was slightly higher in the presence of microorganisms. The amount of nutrients leached from cedar needles was consistent from one month to the next; this likely provided microorganisms with a steady supply of heterotrophic energy possibly in the form of protein-like fluorescence as observed from the EEMs.
In the incubation treatments without microorganisms, aromaticity decreased, autochthonous character increased, and molecular weight decreased. Together, these characteristics suggested a DOM breakdown of the aromatic portion, leaving behind a DOM pool that was more autochthonous in character and lower in molecular weight. The effects of microorganisms were also similar in that aromaticity and autochthonous character decreased while molecular weight increased. As was the case in the previous month, these parameters pointed to the microbial use of the labile portions of DOM and the aromaticity decreased possibly due to the further breakdown of the aromatic DOM pool as an additional resource. From the EEMs, the fulvic-like fluorescence appeared to have two fluorescing centres instead of the usual one after the incubation period, implying that microorganisms in the water column were responsible for the change in the nature of this fluorescence.

**Biofilm as a DOM Source**

Both incubation treatments with biofilm in September decreased DOC concentrations slightly, suggesting an uptake of organic carbon by the biofilm. In the incubation without microbes, the increase in aromaticity, decrease in autochthonous character, and increase in molecular weight all pointed to a use of the labile, DOM components. Microorganisms in the water column essentially had no effect on autochthonous character or the molecular weight but increased aromaticity slightly. However, the aromaticities from the two incubation treatments were within the margin of error of each other, and therefore could be interpreted as no change.

The EEMs from the incubation treatments with microorganisms indicated a protein-like fluorescence that decreased after the incubation period. However, this did not imply that biofilm was a source of this fluorescence because it was not observed in the incubation without microorganisms. Instead, it appeared that microorganisms in the water column mobilized labile DOM from the biofilm and subsequently assimilated it.

In October, DOC concentrations did not change. The incubation without microorganisms was similar to the previous month as the parameters implied a use of the labile DOM portion. Observations from the EEMs also supported this as the small amounts of protein-like fluorescence disappeared after the incubation period. The effect of microorganisms decreased
aromaticity, decreased autochthonous character, and increased molecular weight. Again, the latter two variables implied that microorganisms used the labile DOM portion and perhaps degraded the aromatic DOM pool as an additional resource, resulting in a decrease in aromaticity. However, this was not observed in the EEMs as the humic-like fluorescence actually increased after the incubation period.

Biofilm was not a significant source of DOC in November. From the incubation without microorganisms, the DOM decreased in aromaticity and molecular weight while it increased in autochthonous character. These parameters, together, implied a break up of the aromatic portion of the DOM, as this would have resulted in a more autochthonous DOM pool that was lower in molecular weight. However, no change was observed in the EEMs as only small amounts of fulvic- and humic-like fluorescence were detected.

When microorganisms were added, the DOM was more aromatic, less autochthonous in character, and higher in molecular weight. As in October, this indicated microbial use of the labile DOM pool. However, the aromaticity increase could be interpreted as the sufficiency of the labile DOM as a microbial resource, thus the aromatic portion was not used. The EEMs showed that fulvic- and humic-like fluorescence increased after the incubation period and this supported the result of an unused recalcitrant and aromatic DOM pool.

Microorganisms in the Water Column as a DOM Source

In September, protein-like fluorescence was observed in both types of incubations at the beginning of the incubation period, suggesting a biofilm source. But this fluorescence was stronger in the incubation with microorganisms, which was likely the result of the microbial inoculum. The decrease in fluorescence after the incubation period suggested a degradation of the DOM regardless of the presence of microorganisms.

The EEM results from the October samples treated with a microbial inoculum showed lower fulvic- and humic-like fluorescence compared to the one without microorganisms, suggesting a microbial assimilation of these DOM components at the start of the incubation
period. The decreased fluorescence after the incubation period implied a degradation process that occurred regardless of the presence of microorganisms from the water column.

In November, the EEMs were similar in both incubation treatments and after the incubation period. This suggested that biofilms at the beginning at the incubation period were not a significant source of protein-like fluorescence, perhaps indicating a shift in the community structure. In general, autochthonous sources did not play an important role in determining DOM composition in late autumn. Overall, photobleaching of DOM (i.e., Moran and Covert, 2003) was not suspected to be a major factor, as the conditions in the environmental room did not use a natural light simulator.

In general, we expected microorganisms to increase DOC concentrations more significantly than was observed in the study. Although air-drying leaf litter for 24 hours before leaching can change the amount of nutrients leached (Gessner, 1991), this procedure was required to standardize the dry mass of leaves used in the incubations (see McArthur and Richardson, 2002). Also, there may have been some differences within the incubations resulting from the possible presence of fungi and microorganisms on the leaf surface.

The dendrogram implied that chemical characteristics such as aromaticity, molecular weight, and spectral slopes were determined mainly by DOM source. This lends support to the working hypothesis that vegetation species affects DOM quality and quantity (Jaffé et al., 2008). The strong correlations between SUVA$_{280}$, E2/E3 ratios, and S supported what is currently understood about DOM. A decreasing autochthonous character was correlated with increasing aromaticity, which from a diagenetic perspective (Sinsabaugh and Foreman, 2003), suggested a microbial use of the DOM portions that are structurally less complex and lower in molecular weight. An increasing autochthonous character was also correlated with an increase in E2/E3 ratios (or decrease in molecular weight), which implied DOM breakdown to either more labile DOM, small, refractory DOM from cellular releases (Sinsabaugh and Foreman, 2003; Bertilsson and Jones, 2003), or microbial use of the recalcitrant, aromatic components. Increasing aromaticity was also correlated with decreasing E2/E3 ratios (or increase in molecular weight) which was plausible as aromatic DOM is thought of as being heavier than the smaller, non-aromatic portions (Sinsabaugh and Foreman, 2003). Although aromaticity was observed to
sometimes decrease when microorganisms were added to the incubations, the correlation implied that the relationship between aromaticity and molecular weight in general was still valid.

Conclusion

DOM isolated from allochthonous watershed sources (birch and cedar leaf litter) and an autochthonous source (biofilm) was examined using UV and fluorescence spectroscopy. In terms of our first hypothesis, the amount of DOC leached from deciduous leaf litter was the highest but it decreased as autumn progressed. The consistent increase of DOC each month from coniferous needles possibly acted as a stable source of heterotrophic energy. As expected, biofilms had almost no effect on concentrations. In terms of our second hypothesis, DOM characteristics were indeed determined mainly by DOM source but microorganisms in the water column mainly affected DOM characteristics, not DOC concentrations. Often, variables measured using UV spectroscopy indicated the microorganisms' preferential use of the labile DOM pool and the occasional use of the aromatic, more recalcitrant pool as a secondary resource. From the EEMs, birch litter was a source of protein-, fulvic-, and humic-like fluorescence that decreased after incubation with microorganisms. Cedar litter was an important source of protein-like fluorescence, which did not appear to result from microbial degradation from aquatic microorganisms. Conversely, EEMs from incubations with biofilm indicated a decrease in overall fluorescence while those containing only surface water showed some apparent degradation that proceeded in the absence of microorganisms. The ubiquity of DOM in aquatic ecosystems and its heterogeneous structure cause some difficulty in linking it directly to its sources – the manipulative study was unable to explain the corresponding field observations in the fall of 2007. However, the sources we examined represented only the most temporally important ones in the study stream and were by no means exhaustive. Nonetheless, our results indicate the need to characterize the finer dynamics that shape DOM in order to better understand this compartment of the global carbon cycle.
### Tables

Table 3.1. Spearman rank correlation coefficients between DOC concentrations and measures of DOM character using UV spectroscopy.

<table>
<thead>
<tr>
<th></th>
<th>Δ SUVA</th>
<th>Δ E2/E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ SUVA</td>
<td>-</td>
<td>-0.275</td>
</tr>
<tr>
<td>Sept</td>
<td>Δ S</td>
<td>-0.056</td>
</tr>
<tr>
<td></td>
<td>Δ DOC</td>
<td>0.490*</td>
</tr>
<tr>
<td></td>
<td>Δ SUVA</td>
<td>-</td>
</tr>
<tr>
<td>Oct</td>
<td>Δ S</td>
<td>0.194</td>
</tr>
<tr>
<td></td>
<td>Δ DOC</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td>Δ SUVA</td>
<td>-</td>
</tr>
<tr>
<td>Nov</td>
<td>Δ S</td>
<td>-0.528**</td>
</tr>
<tr>
<td></td>
<td>Δ DOC</td>
<td>-0.150</td>
</tr>
</tbody>
</table>

* and ** indicate significance at p ≤ 0.05 and 0.01, respectively.
Figures

Figure 3.1. Map of study site (denoted by the star) near Guelph, Ontario, Canada.
Figure 3.2. Illustration of the experimental design; 4 incubation treatments (n = 3) were set up on Day 0 with 0.22 µm filtered stream water. Unfiltered stream water was added to the set, labelled “+ microbes” while 0.22 µm filtered stream water was added to the set labelled “- microbes”. DOC concentrations and DOM characteristics were measured on samples from Day 1 and Day 7.
Figure 3.3. Changes in DOC concentrations (a – c) and SUVA$_{280}$ (d – f) after the incubation period with birch litter, cedar litter, and biofilm in the presence (black bars) and in the absence (white bars) of microorganisms from the water column. Error bars indicate ± 1 SE.
Figure 3.4. Changes in S (a – c) and E2/E3 ratios (d – f) after the incubation period with birch litter, cedar litter, and biofilm in the presence (black bars) and in the absence (white bars) of microorganisms from the water column. Error bars indicate ± 1 SE.
Figure 3.5. Average-linkage dendrogram of the changes in DOC concentrations, SUVA$_{280}$, S, and E2/E3 ratios.
Figure 3.6. Excitation-emission matrices (EEMs) of DOM containing: birch litter with microorganisms (a, b), without microorganisms (c, d); cedar litter with microorganisms (e, f), without microorganisms (g, h). “F”, “H”, and “P” denote the location of fulvic-, humic-, and protein-like fluorescence, respectively. Plotted with 20 contour lines, fluorescence intensities are in Raman units (R.U.).
Figure 3.7. EEMs of DOM containing: biofilm with microorganisms (a, b), without microorganisms (c, d); stream water with microorganisms (e, f), without microorganisms (g, h). Plotted with 20 contour lines, fluorescence intensities are in Raman units (R.U.).
References


Chapter 4: Thesis Conclusions

Here, the main results from the two studies are summarized, and ideas for future research are outlined.

Summary

Unravelling food webs often involves answering questions such as “who eats what”. While this approach has produced a wealth of literature, the intricate dynamics between dissolved nutrients and microbes that occur at the base of aquatic food webs remain poorly understood. This thesis reports on field and laboratory studies that were aimed at understanding the seasonal nature of dissolved organic matter (DOM) in the hyporheic zone of a temperate stream in southern Ontario, and investigating the effect of microbes on various carbon sources that can enter streams. To characterize these biogeochemical processes on a molecular level, UV and fluorescence spectroscopy techniques were used to examine DOM.

In Chapter 2, DOM in the hyporheic zone was found to display seasonal trends that were more variable during the summer months than in the fall or winter. Although large-scale watershed processes (such as the shedding of leaf litter) changed DOM characteristics, particular in October, DOM sources in both surface and groundwater were spatially – rather than temporally – determined in both downwelling and upwelling zones. The EEMs indicated that DOM leached from cedar litter was a likely source of protein-like fluorescence that was also observed in the fall of 2007, while DOM from birch litter in November was similar to what was generally observed in surface and hyporheic waters for most of the year.

In Chapter 3, DOM leached from birch and cedar litter, and biofilm were characterized in the fall of 2007, but could not explain field observations of surface waters, suggesting that DOM in the study stream was under the control of factors not tested here. Nonetheless, the results conformed to expectations in showing that birch and cedar litter were important sources of organic carbon; DOC increases in birch litter incubations were higher at the beginning of the season whereas they were constant in the incubations containing cedar litter. In all the incubation treatments tested, microorganisms from the water column did not appear to be crucial in
increasing DOC, but they did appear to utilize the labile portions of DOM before degrading the recalcitrant portions as an additional resource. Biofilm was not a significant source of DOC, but was a source of protein-like fluorescence at the beginning of the fall.

Since hyporheic zones are biogeochemically unique ecotones beneath streambeds, understanding DOM quality over space and time in these areas lends insight into the resources that shape microbial diversity and community structure. While streamside vegetation is assumed to largely influence DOM quality, the relationships between leachates of known watershed sources and microorganisms in the water column have not been previously studied. In this thesis, detrital energetics was investigated at the molecular level using analytical chemistry techniques that, hopefully, will form the basis of further research that seeks to improve our understanding of the interfaces between terrestrial and aquatic ecosystems.

**Directions for Future Research**

Two limitations of this work should form the basis for future research. The first limitation is that the incubations did not include stream sediment. Since sediment can adsorb DOC from the water column (Lock and Hynes, 1976), the effect of sediment on DOM characteristics warrants further investigation. The second limitation is that the effects of photoreactions were not tested. Since UV can help breakdown aromatic, recalcitrant DOM into more labile DOM (Moran and Covert, 2003), future laboratory or field studies should include natural sunlight as a variable.

**References**


Appendices

Appendix I. Excitation-emission matrices (EEMs) of DOM in the hyporheic zone 2007-2008.
Figure A-1. EEMs of surface and downwelling zone in September and October 2007. “F”, “H”, and “P” indicate locations of the fulvic-, humic-, and protein-like fluorescence. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-2. EEMs of the upwelling zone in September and October 2007. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-3. EEMs of surface waters in January 2008 (a) and February 2008 (b). Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-4. EEMs of surface and downwelling zone in April and May 2008. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-5. EEMs of the upwelling zone in April and May 2008. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-6. EEMs of the downwelling zone in July and August 2008. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-7. EEMs of the upwelling zone in July and August 2008. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-8. EEMs of surface and downwelling zone in September and October 2008. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-9. EEMs of the upwelling zone in September and October 2008. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-10. EEMs from November 2008. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Appendix II. Excitation-emission matrices (EEMs) of source and microbial manipulations in September and November 2007.
Figure A-11. EEMs of incubations with birch litter (a – d) and cedar litter (e – h) in September 2007. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-12. EEMs of with birch litter (a – d) and cedar litter (e – h) in November 2007. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-13. EEMs of with biofilm (a – d) and surface water only (e – h) in September 2007. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Figure A-14. EEMs of with biofilm (a – d) and surface water only (e – h) in November 2007. Plotted with 20 contour lines; fluorescence intensities are in Raman units (R.U.).
Appendix III. MATLAB code

The following MATLAB code (with extension “.m”) was developed in cooperation with Hien Goi (Graduate Student, Rogers Department of Electrical and Computer Engineering, University of Toronto) as part of the Software Carpentry Course (CSC2125) offered in Winter 2008 by Dr. Greg V. Wilson (Faculty, Department of Computer Science, University of Toronto).

Modern scientific instruments are usually computer-controlled by bundled propriety software. While this software is often user-friendly, it is inflexible and does not allow advanced users to manipulate or manage data to suit their specific needs. Therefore, several custom codes were written in MATLAB in order to automate the workflow of generating the excitation-emission matrices from fluorescence and UV spectroscopic data. MATLAB was chosen as the platform of choice to write this code because it is easy to disseminate to other scientists, and is a relatively simple programming language that allows users to change the code as needed.

There are 4 parts to the code: “Workflow1.m”, “ModifyCzir.m”, “CreateMatrices.m”, and “CreatMatricesHelper.m”. The first code is used to generate the contour plots for the excitation-emission matrices (EEMs), but the other codes were written separately and linked to the main code to perform functions related to “Workflow1.m”. Therefore, all parts of the code are required to generate the the EEMs in MATLAB.
Workflow1.m

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Main Routine Part 1:
% - read in data files
% - create matrices from data
% - plot contour of the orginal Czir matrix
% - write modified Czir matrix to csv file
%
% To use this routine
% - modify the name of the workspace to be saved
% - modify the partial and full paths to desired files
% - modify Raman Area constant
% - change name of matrices created to desired names
% - modify the wavelength values of the contour plot to be blanked
% - F5 to execute
%
% Partial paths include the file name without the last 6 characters, i.e.,
% excluding 001.sp, e.g., '[file path]\PonyO' where PonyO001.sp,
PonyO002.sp, ..., PonyO022.sp are the names of the data files.
% File paths may be relative to Workflow1.m or absolute paths
%
% NOTE: files paths for Windows and Macs are DIFFERENT. MATLAB on Windows
% allow both \ and / separate folders while MATLAB on Macs require /.
%
% NOTE: for regular use, only modify the workflow files; do not modify
% function files.
%
% Updated: March 27th, 2008
% Authors: Jessica Wong and Hien Goi
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Clear the workspace
clear;

% Name of workspace to be saved
WorkspaceName = 'DW20(Oct2007).mat';

% Partial path to fluoroscence data files
% e.g. 'DW20(Oct2007) November 9 2007/D200c'
FluoroFiles = 'DW20(Oct2007) May 23 2008/D200';

% Partial path to nanopure data files
% e.g. 'Nanopure November 9 2007/NANOE'
NanoFiles = 'Nanopure May 23 2008/NANO';

% Full file name of abs data file
% e.g. 'DW20(Oct 07) November 13 2007.txt'
AbsFile = 'DW20(Oct07) April 12 2008.txt';

% Full file name for modified Czir matrix with extension .csv
% e.g. 'ModifyCzir.csv'
ModifiedCzirFile = 'DW20 Oct 2007.csv';

% Raman Area constant
RamanArea = 11000;

% Specify the corresponding wavelength values of the contour plot to remove
% instrument artifacts. Will need to run Workflow1 once first to see the
% Original contour plot to be able to specify the exact wavelength values. 
% See ModifyCzir.m for more details

% Wavelengths to identify the upper left corner of the contour plot to be
% blanked
UpperYWaveLength = 320;
UpperXWaveLength = 400;

% Wavelengths to identify the lower right corner of the contour plot to be
% blanked
LowerXWaveLength = 450;
LowerYWaveLength = 300;

% Call CreateMatrices to create the 6 matrices and 1 vector
% Modify output names as desired, e.g.
% [FluoroMat FluoroMatRaman FluoroMatRamanCorr NanoMat NanoMatRaman Czir Abs]
% [FluoroMat FluoroMatRaman FluoroMatRamanCorr NanoMat NanoMatRaman Czir Abs] =
% ... CreateMatrices(FluoroFiles, NanoFiles, AbsFile, RamanArea);

% For regular use, do not modify the below section.
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Remove instrument artifacts located the upper left and lower right
% corners of the Czir matrix and zero any negative values
ModifiedCzir = ModifyCzir(Czir, UpperYWaveLength, UpperXWaveLength,
LowerXWaveLength, LowerYWaveLength);

% Write the Czir matrix for later use in Workflow2
dlmwrite(ModifiedCzirFile, Czir);

% Define the axes for the contour plot
Em = [300:0.5:550.5];
Ex = [210:10:420];

% Close all previous figures
close all;

% Plot original contour
figure;
contour(Em, Ex, Czir, 20);

% Plot modified contour
figure;
contour(Em, Ex, ModifiedCzir, 20);

% Save workspace.
save (WorkspaceName);

% Clear the temporary variables (add more variables to clear as desired)
clear WorkspaceName
function oModifiedCzir = ModifyCzir(iCzir, iUpperYWave, iUpperXWave, iLowerXWave, iLowerYWave)

% Czir is a 22x502 matrix. The corresponding axis wavelengths are shown in % the diagram below.

% The below code will loop the matrix from row 1, column 1 across to row 1, % column 502, then up to row 2, column 1 across to row 2, column 502 and so % on until row 22, column 502. As we loop through the matrix, we will zero % all negative entries and remove the instruments artifacts located in the % upper left and lower right triangular regions by writing NaN's to those % cells. Those regions are specified by the user who passes in % iUpperYWave, iUpperXWave, iLowerXWave, and % iLowerYWave, which by the diagram uniquely identify those regions. % Note that X wavelengths correspond to the columns of the matrix and Y % wavelengths correspond to the rows of the matrix.

% Calculate the corresponding rows and columns based on the specified % wavelengths. The equations on the right hand side are realized by % considering the linear mappings from the X wavelengths to the columns and % the Y wavelengths to the rows % e.g. for the rows, let f(Y) = mY + b be the mapping of a Y wavelength to % row f(Y). Therefore f(210) = 1 and f(420) = 22. Plug these points into % f(Y) = mY + b, we get % Eqn (1): 1 = m(210) + b and % Eqn (2): 22 = m(420) + b % Subtract Eqn (1) from Eqn (2), we get 21 = m(210) => m = 1/10 % Sub m back into Eqn (1), we get b = -20. % Hence, f(Y) = (1/10)Y - 20. or (Y-200)/10 which is the equation used
% below. A similar formula can be derived to relate the X wavelength to
% its corresponding column.
UpperRow = (iUpperYWavelength - 200)/10;
UpperCol = (iUpperXWavelength - 300 + 250.5/501)*501/250.5;
LowerCol = (iLowerXWavelength - 300 + 250.5/501)*501/250.5;
LowerRow = (iLowerYWavelength - 200)/10;

% Calculate the columns increments required to generate the triangular
% blanked regions (by writing NaN's)
% Increments = # of columns to be blanked/# of rows to be blanked
UpperColIncrement = (502 - UpperCol)/(22 - UpperRow);
LowerColIncrement = (502 - LowerCol)/LowerRow;

% Initialize the max column to be blanked for the upper region since
% we are only blanking first few columns in the upper region and we need to
% increment the max column as move up the matrix row by row
% A max column is not needed for the lower region because we are blanking
% for the lower column (LowerCol) to the last column of the matrix (502).
% However, we do need to increment the LowerCol as we move up the matrix
% row by row to create the triangular shape
UpperMaxCol = UpperColIncrement;

% Loop through the entire matrix
for i = 1:22
  for j = 1:502
    % If the cell is negative, zero it
    if (iCzir(i,j) < 0)
      iCzir(i,j) = 0;
    end

    % If within the region specified to be blanked out, replace all
    % numbers in this region with NaN (regardless of whether it is 0)
    if (i >= UpperRow && j <= UpperMaxCol)
      iCzir(i,j) = NaN;
    end

    if (j >= LowerCol && i <= LowerRow)
      iCzir(i,j) = NaN;
    end
  end

  % If we're above the upper row to be blanked, increment the upper max
  % column to be blank to create the triangular shape as we move up the
  % matrix row by row
  if (i > UpperRow)
    UpperMaxCol = UpperMaxCol + UpperColIncrement;
  end

  % Increment the lower column to be blanked to create the triangular
  % shape as we move up the matrix row by row
  LowerCol = LowerCol + LowerColIncrement;
end

oModifiedCzir = iCzir;
function [oFluoroMat oFluoroMatRaman oMatRamanCorr oNanoMat oNanoMatRaman oCzir oAbs] = CreateMatrices(iFluoroPartialPath, iNanoPartialPath, iAbsPath, iRamanArea)

% Create fluoroscence matrix
oFluoroMat = CreateMatricesHelper(iFluoroPartialPath);

% Create nanopure matrix
oNanoMat = CreateMatricesHelper(iNanoPartialPath);

% Divide entries by Raman Area constant
oFluoroMatRaman = oFluoroMat/iRamanArea;
oNanoMatRaman = oNanoMat/iRamanArea;

% Create oMatRamanCorr matrix
oMatRamanCorr = oFluoroMatRaman - oNanoMatRaman;

% Create abs vector
oAbs = dlmread(iAbsPath, '', 8, 8);

% Create IFC matrix
ExAbs = oAbs(22:20:442,:);
EmAbs = oAbs(201:1:702,:);
for i = 1:length(EmAbs)
    for j = 1:length(ExAbs)
        IFC(i,j) = ExAbs(j) + EmAbs(i);
    end
end

% Create Czir matrix
Czir = oMatRamanCorr.*10.^exp(0.5*IFC);

% Output the transpose of Czir
oCzir = Czir';
function oMatrix = CreateMatrixHelper(iPartialFileName)

% Initiate the output matrix to blank
oMatrix = [];

% Range of data to read in: row 54 to 555 and column 1 to 1.
Range = [54 1 555 1];

% Loop through the 22 files to create the output matrix
% Note: can make 22 an input parameter also if it's not always 22 files
for i = 1:22
    % When i is less than 10, add an extra 0 to fill out the file name
    if i < 10
        % Filename is made of the input partial path concatenated with
        % 0 or 00 and the string version of i and .sp
        FileName = [iPartialFileName '00' int2str(i) '.sp'];
    else
        FileName = [iPartialFileName '0' int2str(i) '.sp'];
    end

    % Read in the matrix column from data file
    TempCol = dlmread(FileName, '', Range);

    % Append the column to the end of the output matrix
    oMatrix = [oMatrix, TempCol];
end