# Dissolved Organic Matter in the Anthropogenically Impacted Grand River and Natural Burnt River Watersheds

by

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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Science
in
Earth Sciences

Waterloo, Ontario, Canada, 2011

# AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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# Abstract

Dissolved organic carbon (DOM) is one of the largest cycled organic carbon pools on Earth and an important biogeochemical factor in aquatic systems. DOM can act as an energy source for microorganisms, alter the depth of the photic zone for photosynthesis, absorb harmful ultraviolet radiation, as well as alter the transport and toxicity of contaminants.

The purpose of this research project was to characterize DOM in the Grand River watershed in Ontario, Canada using a wide range of qualitative and quantitative techniques and determine the impact of anthropogenic activities as well as seasonal and longitudinal changes on DOM processes.

To reach the study objectives, historical data was analyzed to determine the seasonal cycle in the Grand River watershed. Intensive longitudinal sampling surveys were undertaken to evaluate the DOM characteristics and processes in the Grand River. Surveys of the less impacted Burnt River watershed were used as a comparison watershed to the Grand River to evaluate allochthonous and autochthonous indicators of DOM source and human impacts on DOM processes. Drinking water surveillance data was used to evaluate the effect of DOM in the Grand River on formation of disinfection by-products (DBPs).

Different trends were seen in the Grand River in terms of longitudinal area and season. The headwaters of the river showed more autochthonous DOM in the spring and winter compared to the fall and summer. The lower-central river peaked in autochthonous DOM in the summer and was more allochthonous in the winter. DOM generally became more autochthonous downstream in the Grand River and was most autochthonous below the large sewage treatment plants (STPs) in the central portion. Protein content, measured as protein-like fluorescence normalized to DOC concentration, was strongly related to $\delta^{15}$N of DON; both are associated with autochthonous DOM in the Grand River and show the effects of the major STPs. The increase in autochthonous DOM below the STPs is likely associated with nutrient enrichment stimulating primary production and macrophyte growth.

Based on the comparison of the Burnt River with the more impacted Grand River, the effect of lakes and photodegradation can make discrimination of autochthonous and allochthonous DOM more difficult. The ratio of DOC/DON and protein-like fluorescence proved to be robust indicators despite photodegradation. Human impacts on the Grand River watershed

result in a greater seasonal cycle, high primary production in the summer and a downstream trend of increasing autochthonous DOM compared to the Burnt River.

Based on drinking water surveillance data and literature review, autochthonous DOM caused greater DBPs in the drinking waters fed by the Grand River. This is currently a threat to human health and DBPs in sewage treatment plant effluent may be a threat to ecosystem health

# Acknowledgments

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# Chapter 1: Introduction and Site description

## 1.1 DOM and Rivers

As one of the largest cycled organic carbon pools on Earth, dissolved organic matter (DOM) is an important biogeochemical factor in rivers (Amado et al. 2006). DOM absorbs harmful ultraviolet radiation, alters the toxicity and transport of contaminants, acts as an energy and nutrient source for microorganisms and changes the depth of the photic zone for photosynthesis (Hood et al. 2003). In rivers, an excess of bioavailable organic matter can cause high rates of oxygen consumption (Sand-Jensen and Pedersen 2005). Low oxygen concentrations are a treat to ecosystem health and lead to fish morality (Eyre et al. 2006). In drinking waters, DOM can cause issues with taste and odour as well as form harmful disinfection by-products (DBPs) during treatment (Volk et al. 2002).

DOM can be produced within the aquatic environment by the microbial community or primary producers such as phytoplankton, periphyton and macrophytes and is termed autochthonous. Autochthonous DOM is generally largely bioavailable and contains a high proportion of organic nitrogen. Visual and UV absorbance is low and protein content is high compared to terrestrial DOM (Alber and Valiela 1994, Hood et al. 2005, Mann and Wetzel 1996).

DOM introduced from the terrestrial environment mainly originates from the upper soil zone and is known as allochthonous (Agren et al. 2008). Allochthonous DOM contains a high proportion of refractory lignin from the breakdown of terrestrial plants. The aromatic carbon rings of lignin act as a biomarker and increase the UV absorbance of DOM (Duan et al. 2007a, Hood et al. 2005).

The river continuum concept is a frame work for natural river systems which separates large rivers into three sections: headwaters, medium-sized streams and large rivers. In the headwaters, the stream is shaded by riparian vegetation which reduces autotrophic production and contains mainly allochthonous OM. In medium-sized streams, increased size from the headwaters causes a shift from solely terrestrial inputs of organic matter to autochthonous primary production. In large rivers, fine particulate organic matter from upstream processing

increases turbidity, limiting primary production and resulting in heterotrophy and allochthonous OM (Vannote et al. 1980).

## 1.2 Site Description

### 1.2.1 Grand River Watershed

The Grand River watershed is the largest watershed flowing into the north side of Lake Erie and the largest in southern Ontario (fig. 1.1). It has a drainage area of 6800 km$^2$ and a length of approximately 300 km. The major tributaries of the Grand River are the Conestogo, Speed and Nith Rivers. The watershed is 93% rural and 7% urban with a population of 950 000. Most of the population, 500 000, is concentrated in the urbanized central watershed in Waterloo, Kitchener, Cambridge, Brantford and Guelph. Growth in the watershed is expected to increase the population within the watershed by 57% or 443 000 people by 2031 (Ministry of Public Infrastructure Renewal 2006).

The watershed is dominated by glacial deposits underlain by Paleozoic bedrock made up of limestones and dolostones. The Paleozoic bedrock in the watershed ranges from Upper Ordovician to Middle Devonian dipping slightly to the west and is part of the Michigan Basin (Morgan and Karrow 2004). The limestones and dolostones in the underlying bedrock contribute to the water chemistry. Dissolved bicarbonate contributes to water hardness throughout watershed adding buffering capacity.

Surface deposits in the watershed were associated with the last glacial retreat 15 000 to 12 500 years ago. The northern section of the watershed is dominated by the Huron -Georgian Bay lobe till plain. These tills have low permeability and are poorly drained.  The central portion is a combination of Ontario and Erie lobe till plains and end moraines. The sediments in this area are generally sand and gravel and are relatively permeable. Consequently, infiltration is high and runoff is low in this portion of the watershed. In the lower section of the basin, glacial lake deposits dominate. A lake plain made up of silts and clays characterizes the landscape causing infiltration to be low and runoff high (Karrow and Morgan 2004).

### 1.2.2 The Burnt River Watershed

The Burnt River watershed is located in south-central Ontario, has an area of approximately 1300 km$^2$ and 100 km in length, and flows into Cameron Lake (Fig 4.1, 4.2). The watershed is home to less than 20 000 permanent residents and has a trophic status of

oligotrophic to mesotrophic. The bedrock underlying the Burnt River is carbonate metasedimentary allowing for a higher pH and buffering capacity than many watersheds in south central Ontario (Quinlan et al. 2003).

## 1.3 Research Objectives

The primary goal of this thesis is to characterize DOM in the Grand River watershed in terms of source, fate and transport and the relationship to ecosystem and human health. This was accomplished through measurement of DOM properties as well as historical data records. Four main objectives were completed to reach this goal.

The first objective was to establish seasonal and longitudinal trends over a longer time scale. This was done using provincial water quality monitoring network PWQMN data with monthly monitoring of DOC and DON for seven sites in the watershed from 1981 to 1988. Analysis of these trends allowed for planning of the intensive sampling to capture the full range in seasonal variability.

The second objective was to characterize DOM properties in the Grand River watershed seasonally and longitudinally at high spatial and temporal resolution to determine the importance of autochthonous and allochthonous DOM. This was done with four intensive sampling surveys covering all seasons and 23 sampling sites. Intensive sampling included visual and UV absorbance, 3D fluorescence, size analysis and stable isotopes ($\delta^{13}$C and $\delta^{15}$) properties of DOM which have been shown to be useful in determination of autochthonous versus allochthonous source (Her et al. 2004, Hood et al. 2003, Hood et al. 2005, Mcknight et al. 1991, McKnight et al. 2001, Nguyen et al. 2010). Therefore, the downstream and seasonal evolution or change in DOM source can be determined.

The third objective was to examine the change in DOM characteristics in an impacted river versus a less impacted river to compare measures of allochthonous and autochthonous indictors of source. This was done through the direct comparison of the human impacted Grand River watershed with the less impacted Burnt River watershed. Therefore, the impact of agricultural and urban land use on DOM properties can be examined.

The fourth objective was to examine the implications of DOM properties on ecosystem and human health. This was done by examining disinfection byproducts (DBPs) literature and in

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the drinking water surveillance program (DWSP) within the Grand River watershed. Therefore, the impacts of treatment of autochthonous versus allochthonous DOM can be investigated.

# Chapter 2: Historical Dissolved Organic Carbon and Dissolved Organic Nitrogen export in the Grand River Watershed

Summary

Long-term export of DOC and DON was investigated from 1981 to 1987 for seven subcatchments in the agricultural Grand River watershed in Ontario, Canada. DOC concentration was highest in summer and autumn. DON concentration was highest in the summer and lowest in the winter and increased with the proportion of farmland. Export of DOC and DON per area were higher in subcatchments with greater agricultural land. DOC/DON showed different seasonal trends between the headwaters and lower reaches of the river. The headwaters had high DOC/DON in the summer/autumn and lower in the spring/winter while the lower river had high DOC/DON in the winter and lower in the spring. Differences in discharge and primary production are likely responsible for these trends.

## 2.1 Introduction

Dissolved organic matter (DOM) is an important energy source in aquatic systems as well as a factor in contaminant transport and toxicity. Until recently, the significance of dissolved organic nitrogen (DON) within the DOM pool and nitrogen budget was not well recognized. As a result, few long term export studies have examined DON and DOC/DON in riverine environments (Mattsson et al. 2005). Higher DOC/DON is generally indicative of allochthonous inputs and lower autochthonous DOM production. The ratio of DOC/DON may provide important insight into the seasonal and long-term trends in allochthonous DOM inputs and autochthonous DOM production. Understanding the seasonal variation in the DOM in the watershed is essential to the development of more detailed investigations into the factors that control changes in DOM quality and sources. Little research has focused on the effects of agriculture on DOM sources and quality. A recent study of 34 watersheds in Southern Ontario showed that as the cropland to wetland ratio increased the amount of autochthonous DOM increased (Wilson et al. 2009). Similarly, a study of 86 Finnish watersheds showed an increase in agricultural area from 5% to 30% decreased the DOC/DON by half. Conversely, an increase in peatland area increased the DOC/DON (Mattsson et al. 2005).

In addition to land use, landscape characteristics can affect DOC export. Drainage has an impact on DOC concentration; poorly drained soils have increased DOC concentration regardless of proportion of agricultural land (Wilson and Xenopoulos 2008). The headwaters of the Grand River watershed consist of relatively flat till plains which are high in clay and consequently poorly drained. The centre of the watershed is made up of glacial and outwash features of gravel, sand and silt and is hilly resulting in a high rate of infiltration (Karrow and Morgan 2004). These differences in drainage may have an effect on DOC export within the watershed.

The main objectives of this study are to use historical data from the 1980s to investigate: 1) DOC and DON export in the Grand River, 2) Seasonal and longitudinal changes in DOC concentration, DON concentration, and the ratio of DOC/DON in the Grand River.

## 2.2 Methods

### 2.2.1 Study Area

The 7 sites used in this chapter are shown in Figure 2.1. Refer to Chapter 1 for comprehensive watershed description.

### 2.2.2 Data Analysis

Provincial water quality monitoring network (PWQMN) data was obtained from the Ontario Ministry of the Environment for the period 1970 to 2009. The DOC concentration data was most complete during the period 1981 to 1988 with samples collected monthly and was therefore utilized to provide insight into seasonal changes. Seasons were divided by date: spring March 21$^{st}$ to June 20$^{st}$, summer June 21$^{st}$ to Sept 20$^{th}$, fall Sept 21$^{st}$ to Dec 20$^{th}$, and winter Dec 21$^{st}$ to March 20$^{th}$. DOC concentrations were determined by high temperature catalyzed combustion with infra-red spectrophotometric measurement of $CO_2$ (Ontario Ministry of the Environment 1975). DON was calculated from the difference of total Kjeldahl nitrogen (TKN) and ammonium ($NH_4^+$). Ratios of DOC/DON were calculated as molar ratios.

Precipitation and temperature data were obtained from the Environment Canada National Climate and Information Archive from the Waterloo Wellington Station due to its central location in the watershed. Discharge data and drainage area were obtained from the Environment Canada Water Survey of Canada for PWQMN sites or within several kilometres upstream or

downstream of established PWQMN sites. Average annual discharge using continuous daily discharge and average sampling date discharge were similar (Table 2.3). Annual load and flux calculations were constructed using eq. 1 after (Hope et al. 1997) using discharge on dates sampled. $load = K \sum_{i=1}^{n}(C_i Q_i / n)$, where K is a conversion factor for period of time, $C_i$ is instantaneous concentration, $Q_i$ is discharge on the day of sampling, n is the number of samples.

Statistical analyses were conducted with SYSTAT (Version 12). Correlations were made using the Pearson correlation with Bonferroni probabilities. Two-sample t-tests assuming unequal variance were used to determine differences between seasons.

## 2.3 Results

Seasonal treads in discharge, DOC concentration and DOC/DON in the upper Conestogo River are shown in Fig. 2.2. DOC concentration increases in the spring and summer and decreases in the fall and winter. DOC was slightly negatively correlated with discharge (r = -0.303, $\rho$ = 0.040, n = 79) and DOC/DON was moderately negatively correlated with discharge (r = -0.560, $\rho$ = 0.000, n = 79). DON was positively correlated with discharge (r = 0.656, $\rho$ = 0.000, n = 79) and showed no correlation with DOC ($\rho$ = 0.814, n = 79).

The lower-central river showed similar trends to the headwaters (Fig. 2.3). DOC concentration showed a cycle of generally increasing in the spring and into the summer then decreasing in the fall and through the winter. However, DOC was not correlated to discharge ($\rho$ = 0.822, n = 75). Like the headwaters DOC/DON was moderately negatively correlated with discharge (r = -0.491, $\rho$ = 0.000, n = 75) and DON was moderately positively correlated with discharge (r = 0.423, $\rho$ = 0.000, n = 75).

Table 2.2 provides a comparison of the two proportions of the watershed based on season. In the headwaters, spring and winter had the highest average discharge over the 7 year period. Conversely, in the lower-central portion of the watershed the spring and the fall had the higher discharge. The average DOC concentration showed a different seasonal pattern in the headwaters compared to the lower-central river. The headwaters had higher DOC concentrations in the summer and lowest in the winter. The spring and fall were not significantly different (Table 2.4) and both were significantly different from winter and summer. In the lower-central river, DOC concentrations in the summer and fall were the highest and not significantly different. Spring and winter had the lowest concentration and were not significantly different

from each other. Accordingly, the summer/fall and spring/winter DOC concentrations were significantly different.

DON concentrations in the headwaters and lower-central river (at Glen Morris) were higher in the spring and summer and lower in the winter. The fall season varied between groups. In the headwaters DON was lower in the fall compared to summer (Table 2.4) but in the lower river fall and summer were not significantly different.

DOC/DON ratios in the two portions of the watershed showed different seasonal trends. However, in both cases DOC/DON was the lowest in the spring time. The spring DOC/DON was significantly different from summer and fall in the headwaters (Table 2.4), but not from the winter. In contrast, in the lower river the spring was only significantly different from the winter and not from summer and fall.

2.3.1 Climate and seasonal trends

Fig. 2.4 shows the variability in annual precipitation and mean annual discharge in the headwaters and reaches of the lower river. Years with higher mean annual precipitation generally have higher mean annual discharge. Mean annual DOC flux in the lower-central river follows the same pattern as mean annual discharge while the headwaters do not.

**2.3.2 DOC and DON exports and downstream trends**

Table 2.3 shows averages and fluxes of DOC and DON for 7 sites throughout the watershed. Discharge and DOC and DON fluxes increase linearly with drainage area (Fig. 2.5). Flux per area of DOC and DON decreased with increasing drainage area, leveling off at drainage areas greater than 2000 $km^2$. Two sites have lower DON flux per area than would be expected for the drainage area, the Speed River and Grand River below Belwood. Both of these sites are downstream of reservoirs, Guelph Lake and Belwood Lake, respectively.

**2.4 Discussion**

**2.4.1 DOC and DON exports**

DOC export is different in different landscapes. The flux per area of DOC in the headwaters and parts of the upper central watershed is higher than those in other parts of the watershed that are not underlain by clay-rich tills. The Speed River has a low flux per area compared to the upper central Grand River with a similar drainage area. This difference is due to the drainage area of the Speed River having thin well drained soils compared to the upper central

Grand River. The lower central Grand River is underlain with glacial outwash features with high infiltration resulting in a comparatively lower DOC flux per area.

A higher percent agriculture increases DON export per area (Fig. 2.6). Fertilization increases soil productivity as well as aquatic productivity which increases DON and decreases DOC/DON in the river. In addition to fertilization, rapid turnover of crops can increase soil DON compared with natural forested or wetland soils. These results are consistent with trends seen in Finnish watersheds (Mattsson et al. 2005).

Decreased DOC and DON per area in the lower central and lower watershed can also be explained by metabolism and photo-mineralization (Wiegner and Seitzinger 2001). The longer residence time of water in the lower portions of the watershed compared to the headwaters allows more time for decomposition, reactions with UV light and sedimentation.

## 2.4.2 Seasonal and longitudinal changes

### 2.4.2.1 DOC Concentration

DOM in the Grand River watershed is strongly influenced by climate and season. In the upper and lower-central reaches of the river, the summer and fall seasons generally have higher mean DOC concentrations compared to the rest of the year. This observation is consistent with a study conducted by Bernal et al. (2005), in a catchment at similar latitude to the lower reaches of the Grand River watershed. The "transitional" season, analogous to the fall region in this watershed, showed the highest DOC concentrations. Although, summer was not studied the "wet" or winter season and "vegetative" or spring seasons had lower concentrations compared to the fall (Bernal et al. 2005). In the Grand River watershed, summer and fall likely have higher mean DOC concentrations due to vegetation death in the fall and turnover of organic matter in the summer after spring growth. Summer and fall seasons also had lower discharge; lack of dilution may account for higher concentrations.

DOC concentration is lower in the lower reaches compared to the headwaters with the exception of Canagagigue Creek. Canagagigue Creek is below a reservoir in an agriculture intensive section of the watershed. Reservoirs, like lakes, increase water residence time allowing for more photo-degradation and microbial metabolism (Amado et al. 2006). Similarly, the lower Grand River has higher residence time than the headwaters allowing for more degradation and reduction in DOC concentration. Landscape differences between the headwaters and central and

lower reaches of the river may affect DOC concentration. DOC concentrations have been shown to be higher in areas of poorly drained soils (Wilson and Xenopoulos 2008). Higher DOC concentrations in the headwaters of the Grand River compared to the lower-central reaches may be due to poorly drained soils in contrast to high infiltration outwash features of the central Grand River (Nelson et al. 2004).

2.4.2.2 DON concentration

Mean DON concentrations were higher in the summer and lower in the winter in the Grand River watershed. This seasonal pattern is similar to seasonal trends observed in Finnish boreal catchments (Mattsson et al. 2005). Mean DON concentrations in the Grand River watershed were higher than Welsh watersheds with similar agricultural land percentage (Fig. 2.8). (Mattsson et al. 2009). This could be due to greater intensification of agriculture per area and greater use of nitrogen containing fertilizer.

DON concentration showed little downstream trend. Concentrations were slightly lower in the upper-central portion but increased in the lower portion below the major wastewater treatment plants. Canagagigue creek had the highest mean DON concentration in this data set. Intensive agriculture and use of nitrogen containing fertilizer in the surrounding area as well as elevated primary production in the upstream Woolwich reservoir may contribute to high concentrations of DON.

2.4.2.3 DOC/DON

DOC/DON ratios provide an indicator of the origin of DOM in the river. Throughout the watershed the mean DOC/DON was quite low (6-13), indicating that DOM was highly autochthonous. Low DOC/DON ratio are consistent with watersheds with greater than 50% agricultural use (Fig. 2.7) (Mattsson et al. 2005). The autochthonous production is caused by input of inorganic nutrients shown by the strong relationship with DOC/DON (Fig. 2.9, 2.10).

Seasonal changes are seen in DOC/DON but differ between the headwaters and lower-central river. Few studies evaluate DOC/DON on such a long-term scale in an impacted freshwater watershed. The study of Finnish river watersheds by Mattsson et al. was 5 years in length and showed higher DOC/DON in the winter and lower in the summer similar to the lower-central Grand River. However, the headwaters showed higher DOC/DON in the fall/summer and lower in the spring/winter. This was similar to small forested stream catchment in the study by

Bernal et al. which showed the fall to have the greatest DOC/DON and in lower winter and spring (Bernal et al. 2005).

DOC/DON decreased downstream in the Grand River watershed. This shows that DOM in the river becomes increasingly autochthonous downstream throughout the 7 year period. This downstream trend was likely due to the cumulative effects of agricultural land use and sewage effluent. Canagagigue Creek was the most impacted site in this data set based on DOC/DON. In terms of nutrients and potential impact the Grand River Conservation Authority rated the Canagagigue Creek below the Woolwich reservoir very high, the highest rating (Nelson et al. 2004). DOC/DON may be an important indicator of ecosystem health which decreases downstream in the Grand River watershed.

## 2.5 Conclusion and Implications

Agriculture in the Grand River watershed greatly influences the DOM dynamics of the river. As the proportion of farmland increases, DOC/DON decreases showing an increase in primary productivity and autochthonous DOM. This type of DOM has implications for river and ecosystem health. Autochthonous DOM is more available to heterotrophic microorganisms and may cause oxygen depletion when consumed. These concerns are particularly important in the lower-central and lower watershed and Cangagigue Creek which are the most impacted. Seasonally, the more impacted lower-central Grand River faces the most stress in the summer when primary production is highest and discharge is at its lowest.

# Chapter 3: Whole watershed seasonal characterization of dissolved organic matter in the Grand River

Summary

Seasonal and downstream trends in dissolved organic matter (DOM) in the impacted Grand River Watershed in Ontario, Canada were investigated during 2007 and 2008. Visual and UV absorbance, 3D fluorescence, size analysis and stable isotopes ($\delta^{13}$ and $\delta^{15}N$) were used to characterize water samples from 23 sites every 20km along 300km of river. Properties of DOM show a downstream increase in the proportion of autochthonous DOM peaking below the large sewage treatment plants (STPs) inorganic nutrient loading. $\delta^{15}N$ of DON showed a strong relationship with protein-like fluorescence normalized to DOC concentration ($R^2 = 0.513$). This correlation shows that the N source for autochthonous DOM is different than that of allochthonous DOM in the Grand River. $\delta^{15}N$ of DON may be a useful indicator of DOM source. In addition to nutrient loading, subsurface geology affected DOM characteristics. Poorly drained soils in the headwaters contribute to higher DOC concentrations and more allochthonous properties. In a large impacted river like the Grand River, DOM characteristics are controlled by complex interactions between land-use changes, subsurface geology, processing in reservoirs, primary production, and microbial/photodegradation. 3D fluorescence and stable isotopes of DOM are effective techniques in understanding these interactions.

## 3.1 Introduction

Dissolved organic matter (DOM) is an important component of the biogeochemistry of rivers. DOM is one of the largest cycled organic carbon pools on Earth (Amado et al. 2006). In riverine ecosystems, DOM is utilized in the microbial food web by supporting bacterial growth and causes oxygen depletion when in excess (Sand-Jensen and Pedersen 2005). In surface waters used for drinking water, DOM can create problems with taste and odour. Through the water treatment process, organic matter can react with disinfection agents to form disinfection by-products (DBPs) which are an emerging human health concern (Volk et al. 2002).

DOM is termed allochthonous when it originates from terrestrial sources and autochthonous when produced within the river. Allochthonous and autochthonous DOM differ in their structural and chemical properties as well as their lability as a bacterial substrate. These sources have implications for the availability to heterotrophic microorganisms, the depth of the photic zone for photosynthesis, attenuation of damaging ultraviolet radiation through the water column and the mobility of harmful contaminants (Hood et al. 2003).

Allochthonous DOM originates primarily from decomposition of organic matter in the upper most soil zone in the terrestrial or riparian environment (Agren et al. 2008). This material comes from the breakdown of terrestrial plants which contain a high proportion of refractory lignin. Lignin contains aromatic carbon rings which can be used as an allochthonous biomarker in DOM (Duan et al. 2007a).

Aquatic primary production is the main source of autochthonous DOM. In rivers, phytoplankton, periphyton and macrophytes are primary producers which exude DOM and decompose *in situ*. Aquatic plants contain a high proportion of organic nitrogen compared to terrestrial plants. Autochthonous DOM will absorb less visual and UV light compared to allochthonous DOM. DOM derived from aquatic primary producers and microbes also contains labile compounds such as proteins (Alber and Valiela 1994, Hood et al. 2005, Mann and Wetzel 1996).

Most studies on the characteristics of DOM have focused on lakes or small streams. Few have focused on large rivers, and many of these studied the lower proportion of large estuaries and used relatively few DOM parameters (Duan et al. 2007). This study uses several techniques to characterize DOM. Visual and UV absorbance, spectrofluorometry, size analysis and stable isotopic ($^{13}$C, $^{15}$N) characterization were done at the watershed scale to determine the source and biogeochemical processes affecting DOM. Large rivers have a high variability of discharge and water quality temporally and spatially. By surveying the entire length of the Grand River, seasonally, this study captures the temporal and spatial changes of DOM in the river.

The Grand River has been greatly impacted by anthropogenic activities. Water control structures are located along the river. The upper portion of the watershed is greatly influenced by agriculture and is underlain by extensive till plains with low permeability sediments (Karrow and Morgan 2004). The upper portion of the watershed is hypothesized to have allochthonous DOM

from runoff of fertile agricultural soils and wetlands as well as autochthonous DOM. This reach may have a mixture of allochthonous and autochthonous DOM.

Intensive urbanization and sewage treatment plant (STP) inputs occur in the middle reaches of the river (Karrow and Morgan 2004). Urban catchments provide less DOM than forested catchments due to removal of vegetation and upper soil horizons. Also, storm water retention ponds increase DOM processing before entering larger channels (Wahl et al. 1996). This reach is hypothesized to have largely autochthonous DOM due to increased production caused by elevated inorganic nutrients and lower allochthonous inputs.

The lower-central watershed has groundwater recharge offering some recovery from upstream anthropogenic impacts. This catchment is mainly underlain by moraines and glacial outwash features that recharge older higher quality groundwater into surface water. This area and the Nith River catchment are underlain by the Upper Silurian Salina Formation, high in the mineral gypsum ($CaSO_4 \cdot 2H_2O$) (Karrow and Morgan 2004). Older groundwater in sulphate containing substrates are low in DOM due to sulphate reduction (Noseck et al. 2009). This area dilutes both DOM and inorganic nutrients originating upstream.

The lower Grand River catchment is predominately agricultural underlain by a clay plain with higher runoff that increases the river turbidity (Karrow and Morgan 2004). This area is hypothesized to have a mixture of allochthonous DOM from runoff and autochthonous DOM produced in the large channel.

The main objectives of this study are 1) to characterize the change in DOM concentrations and characteristics from headwaters to the mouth in different seasons, 2) examine the contribution of allochthonous and autochthonous sources to DOM as indicated by DOM characteristics in a large impacted river, and 3) examine the implications of DOM source for river management of DOM.

## 3.2 Study Site

The characteristics and locations of the study sites are described in detail in Chapter 1.

## 3.3 Methods

### 3.3.1 DOC, TDN, $NO_3^-$, $NH_4^+$ and DON concentrations

Samples for dissolved organic carbon (DOC) concentration, total dissolved nitrogen (TDN), nitrate, ammonia were filtered with a Whatman 0.45 μm syringe-tip after collection.

DOC and TDN concentrations were measured using a Tekmar Dohrman Apollo 9000 high temperature catalytic oxidation TOC analyzer with total nitrogen add-on equipped with an autosampler. Samples were sparged with 20% phosphoric acid to remove any inorganic carbon prior to injection into the catalyst combustion tube. Precision of DOC analysis was typically $\pm 0.2$ mg $l^-$ and TDN analysis was $\pm 0.05$ mg $l^-$.

Nitrate ($NO_3^-$) was measured using a Dionex ICS-90 ion chromatograph with an IonPac AS14A column and AS40 automated sampler. Dionex brand standards were used to make the calibration curve and correct concentrations. Precision for $NO_3^-$ analysis was $\pm 0.05$ mg $l^-$.

Subsamples for ammonium ($NH_4^+$) were acidified to pH 4 with 10% $H_2SO_4$ after filtration. $NH_4$ concentrations were measured using a Technicon Auto Analyzer III colourimetric analyzer. The precision of this analysis was $\pm 0.005$ mg $l^-$.

Dissolved organic nitrogen (DON) was determined through subtraction of dissolved inorganic nitrogen species ($NO_3^- + NH_4^+$) from TDN. C/N ratios of DOM were calculated as molar ratios of DOC/DON.

### 3.3.2 Fluorescence measurements

Water samples for spectrofluorometric 3D emission-excitation matrix (EEM) measurements were filtered through a 0.45 μm cellulose acetate on the day of collection and stored at 4°C. Measurements were made using a Varian Cary Eclipse fluorescence spectrophotometer. Fluorescence intensity was measured for excitation wavelengths from 250-450 nm at 1 nm increments and emission wavelength from 300-600 nm at 10 nm increments. Fluorescence index (FI) was calculated from the ratio of emission intensities at 450 to 500 nm at an excitation of 370 nm (McKnight et al. 2001). Humic-like fluorescence was measured at the peak occurring at Ex/Em 330-380/430-480 nm, and the protein-like fluorescence at Ex/Em 298-305/332-335 nm (Wu et al. 2007).

### 3.3.3 Visual and UV Absorbance

Water samples for absorbance were prepared and stored in the same manner as fluorescence samples. Using a Beckman DU® Series 500 Spectrophotometer, samples were scanned across the visual and UV wavelengths (700-200 nm) for absorbance at 5nm intervals with a 1 cm path length. Nanopure deionized water was used as a blank for absorbance measurements. Specific UV absorbance (SUVA) was calculated using the absorbance at 254 nm

normalized to DOC concentration (Weishaar et al. 2003). SUVA = $A_{254}$ / DOC * 100 (a254 m$^{-1}$/DOC mg l$^{-1}$). Absorbance at 250 nm/365 nm (a250/a365) was also examined as an estimate of the proportion of low-molecular-weight DOM to high-molecular-weight DOM (Amado et al. 2006).

### 3.3.4 High-Performance Size Exclusion Chromatography

Molecular weight was determined as apparent molecular weight (Kohler et al. 2002) by high-performance size exclusion chromatography (HPSEC). HPSEC was performed with a liquid chromatograph (Waters 600s, U.S.A.) with a photodiode array detector (Waters 996) measuring at 254nm. A TSK-gel$^®$ column (7.8 mm × 30 cm and a particle size of 5 µm; model G2500PWXL, Tosohaas, Japan) combined with guard column (TSK-gel$^®$ Guard PWxl, 6 mm x 4.0 cm, particle size 12µm) were used for size exclusion. For each run, 100 µL of sample was injected onto the column with phosphate buffer (0.1 mol l$^{-1}$ NaCl, pH 6.8) as an eluent, and the flow rate was set at 0.5 ml·min$^{-1}$ (Wu et al. 2003). The column void volume and total permeation volume of the column were determined using Blue Dextran (Sigma-Aldrich) and acetone (Sigma-Aldrich), respectively. Sodium polystyrene sulfonates were used as molecular mass calibration standards (1 100, 3 610, 4 800, 6 500, 15 450, and 31 000 Da).

### 3.3.5 Stable isotopic analyses: $\delta^{13}$C and $\delta^{15}$N

Carbon ($^{13}$C/$^{12}$C) and nitrogen ($^{15}$N/$^{14}$N) in DOM was measured on 0.45 µm cellulose acetate filtered samples. The filtrate was first freeze-dried then dialyzed in 10 ml 100Da tubes to remove dissolved inorganic nitrogen and then freeze dried again. Subsamples were run on an Isochrom Continuous Flow Stable Isotope Mass Spectrometer (Micromass) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108) by the Environmental Isotope Laboratory (EIL) at the University of Waterloo, Ontario.

### 3.4 Results

### 3.4.1 Discharge, DOC concentration and DOC load

Discharge throughout the seasons was highly variable and did not follow the typical hydrograph for non-impacted rivers (Fig. 3.1). The June and February surveys had the highest discharge while September and October had the lowest. The discharge in the headwaters did not change significantly between surveys with the exception of the February survey which was higher.

DOC concentrations varied by as much as 2 fold downstream with a general decreasing trend downstream throughout all the seasons (Fig 3.2). Overall, DOC was negatively correlated with distance downstream for all surveys (r = -0.751, $\rho$ = 0.000, n = 82). Correlations between variables during all surveys are summarized in Table 3.2. Throughout all four seasons, the DOC concentrations showed a two step trend downstream. The upper portion of the watershed had higher concentrations and decreased linearly until 143 km for all seasons when excluding the upper most site (y = -0.0511x + 13.019, $R^2$ = 0.7328). Concentrations in the lower river remain relatively constant throughout the seasons between 5 and 7 mg $L^{-1}$.

DOC/DON ratios follow a 2 step trend downstream. For all four seasons DOC/DON ratios decreased linearly until 143 km. In the lower river below 143 km DOC/DON increased linearly downstream until the mouth of the river for the September, October and February surveys. The June survey showed no DOC/DON trend downstream in the lower portion of the river.

### 3.4.2 Nitrogen Concentrations

The dissolved nitrogen species in the river consisted primarily of $NO_3^-$ (0 to 7.1 mg $L^{-1}$) followed by DON (0.24 to 1.6 mg $L^{-1}$) and $NH_4^+$ (0 to 0.59 mg $L^{-1}$) (Fig. 3.3). $NH_4^+$ was only seen in significant quantities (>0.1 mg $L^{-1}$) after the large STPs in the middle reaches of the river. $NO_3^-$ increased downstream from the headwaters (~1 mg $L^{-1}$) peaking after the Kitchener/Waterloo STPs (3.5 to 7 mg $L^{-1}$) and recovering to lower levels in the lower catchment (2 to 4 mg $L^{-1}$). DON remained relatively constant downstream increasing slightly after the influence of STPs. All four surveys show similar downstream trends, however, the magnitude of the $NO_3^-$ concentrations varied between seasons. During all four surveys higher $NO_3^-$ concentrations were associated with lower DOC/DON ratios (Fig. 3.8). The February survey had $NO_3^-$ concentrations two-fold higher than the other surveys. The June, September and October surveys had similar concentrations of nitrogen species.

### 3.4.3 SUVA and FI

Autochthonous DOM has a low SUVA (~1.0 L mg$^-$) and allochthonous DOM has a high SUVA (~7.4 L mg$^-$) (Her et al. 2004). Throughout the surveys, SUVA ranged from 1.2 to 3.7 L mg$^-$ in the Grand River (Fig 3.4). June and October surveys showed decreasing trends downstream. The September and February surveys showed no downstream trend. SUVA was

highest in the February survey, followed by the June and September and lowest in the October survey.

Fluorescence index values showed a great degree of variability in all surveys and ranged from 1.1 to 1.8. Low FI values, ~1.4, indicate terrestrial sources while higher FI values, ~1.9, indicate microbial sources (McKnight et al. 2001). Generally, the lowest FI values were seen in the headwaters (1.1-1.4) and the highest after the major STPs (1.5-1.7). A small positive correlation was observed with downstream distance and FI ($r = 0.361$, $\rho = 0.004$, $n = 87$), a negative correlation with DOC concentration ($r = -0.457$, $p = 0.000$, $n = 87$), and a small negative correlation with DOC/DON ($r = -0.331$, $p = 0.019$, $n = 85$). A small negative correlation between SUVA and FI ($r = -0.381$, $\rho = 0.001$ $n = 87$) was seen.

### 3.4.4 Molecular weight and size

The apparent molecular weight (MW) determined by size exclusion chromatography and an estimate of the proportion of low-molecular-weight to high-molecular-weight bulk DOM, determined by $A_{250}:A_{365}$, are displayed in Figure 3.5. MW ranges from 1060 to 2030 Da and $A_{250}:A_{365}$ ranges from 3 to 13 throughout the surveys. The two measures of MW show a large negative correlation ($r = -0.720$, $\rho = 0.000$, $n = 82$) to each other for all surveys together. MW does not decrease significantly downstream, but a large difference is seen between seasons. September and October had similar MW averaging ~1100 Da. The two surveys with higher discharge, June and February, showed larger MW, ~1200 and ~1800 Da respectively. The ice-covered February survey showed the highest MW.

MW was positively correlated to SUVA ($r = 0.752$, $\rho = 0.000$, $n = 82$), DON ($r = 0.499$, $\rho = 0.000$, $n = 82$) and discharge ($r = 0.533$, $\rho = 0.000$, $n = 82$). $A_{250}:A_{365}$ was negatively correlated SUVA ($r = -0.710$, $\rho = 0.000$, $n = 82$), DON ($r = -0.644$, $\rho = 0.039$, $n = 82$) and discharge ($r = -0.644$, $\rho = 0.000$, $n = 82$). Neither MW nor $A_{250}:A_{365}$ was correlated to FI or DOC/DON ($\rho = 1.0$).

### 3.4.5 Humic-like and Protein-like Fluorescence

Protein-like fluorescence/DOC increased slightly downstream ($r = 0.619$, $\rho = 0.000$, $n = 82$) in all surveys (Fig 3.6). Protein-like fluorescence/DOC ranged from 0.17 to 3.9 rel. fluor. L mg$^{-1}$ and was similar in the June, September and October surveys and lower in the February survey. A small peak in protein-like fluorescence/DOC was seen after the STPs in the middle

section of the river around 150 km from the headwaters. Humic-like fluorescence/DOC had no downstream trend for all surveys. Protein-like fluorescence/DOC was negatively correlated to DOC/DON ($r = -0.435$, $p = 0.008$, $n = 82$) and positively to FI ($r = 0.354$, $p = 0.013$, $n = 85$) and had no correlation to SUVA ($p = 0.679$) or apparent MW ($\rho = 0.081$).

Humic-like fluorescence/DOC varied greatly between seasons and ranged from 2.2 to 10.7 rel. fluor. L mg$^{-1}$. September had the highest average humic-like fluorescence/DOC (8.4 rel. fluor. L mg$^{-1}$), followed by February (6.9 rel. fluor. L mg$^{-1}$), June (5.5 rel. fluor. L mg$^{-1}$) and October (4.4 rel. fluor. L mg$^{-1}$). Humic-like fluorescence/DOC was positively correlated with SUVA ($r = 0.403$, $\rho = 0.010$, $n = 82$) and had no correlation to FI, MW or DOC/DON ($\rho = 1.0$).

Humic-like fluorescence not normalized to DOC concentration was correlated to DOC concentration ($r = 0.588$, $\rho = 0.000$, $n = 82$) and negatively with downstream distance ($r = -0.479$, $\rho = 0.001$, $n = 82$). Humic-like fluorescence was also positively correlated with SUVA ($r = 0.570$, $\rho = 0.000$, $n = 82$) and apparent MW ($r = 0.457$, $\rho = 0.002$, $n = 82$). Unnormalized humic-like fluorescence had a medium negatively correlation with FI ($r = -0.429$, $\rho = 0.009$, $n = 82$). However, humic-like fluorescence was not correlated to $A_{250}$:$A_{365}$ ($\rho = 0.946$) despite its correlation to apparent MW. Unnormalized protein-like fluorescence was negatively correlated with DOC concentration ($r = -0.392$, $\rho = 0.037$, $n = 82$), SUVA ($r = -0.449$, $\rho = 0.003$, $n = 82$), and apparent MW ($r = -0.537$, $\rho = 0.000$, $n = 82$). All four surveys showed higher protein-like fluorescence at lower DOC/DON ratios (fig. 3.9).

Humic-like fluorescence is thought to be indicative of allochthonous DOM and protein-like fluorescence of autochthonous DOM (Wu et al. 2007). The ratio of protein-like fluorescence to humic-like fluorescence (P/HFlu) may provide a useful parameter for the proportion of autochthonous to allochthonous DOM (Fig. 3.7). P/HFlu ranged from 0.06 to 0.52 in all surveys. P/HFlu was similar in the September and October surveys and June and February surveys. P/HFlu was lower in the June and February surveys than the September and October Surveys. P/HFlu is negatively correlated to DOC ($r = -0.568$, $\rho = 0.000$, $n = 82$), SUVA ($r = -0.732$, $\rho = 0.000$, $n = 82$) and apparent WM ($r = -0.700$, $\rho = 0.000$, $n = 82$). P/HFlu was positively correlated with FI ($r = 0.390$, $\rho = 0.040$, $n = 82$).

### 3.4.6 Stable isotopic analyses: δ$^{13}$ Cand δ$^{15}$N

$\delta^{15}$N of DOM (DON-$\delta^{15}$N) in the Grand River ranged from -0.8 to 9.5 and became more positive after the STPs in the middle portion of the river (Fig. 3.10). DON-$\delta^{15}$N was similar in

all four surveys with a similar downstream trend with the headwaters lower (-0.8 to ~4) than the middle river (4 to 9.5) and lower river (2 to 8). DON- $\delta^{15}$N was negatively correlated with DOC concentration (r = -0.634, $\rho$ = 0.000, n = 57) and DOC/DON (r = -0.493, $\rho$ = 0.001, n = 57) and positively correlated to protein-like fluorescence (r = 0.505, $\rho$ = 0.001, n = 56) and protein-like fluorescence/DOC (r = 0.716, $\rho$ = 0.000, n = 56). Individual surveys showed strong a strong relationship between DON- $\delta^{15}$N and protein-like fluorescence/DOC (Fig. 3.12). During the June 2007 survey $NO_3^-$- $\delta^{15}$N and DON- $\delta^{15}$N showed a small positive relationship (Fig. 3.13). DON- $\delta^{15}$N increased at lower DOC/DON during all four surveys (Fig. 3.14).

### 3.4.7 Comparison of autochthonous and allochthonous indicators

SUVA, an estimate of aromaticity, often indicates allochthonous DOM which is generally more aromatic. In all four surveys, SUVA was correlated to normalized humic-like fluorescence and unnormalized humic-like fluorescence, indictors of allochthonous matter (Table 3.2). SUVA was correlated to MW and negatively to $A_{250}$:$A_{365}$. Humic-like measures were also correlated to MW, while unnormalized protein-like fluorescence was negatively correlated with MW.

FI, a common tool used to discriminate autochthonous and allochthonous DOM, showed small positive correlations with protein-like fluorescence/DOC and P/HFlu and negative correlations with humic-like fluorescence, DOC/DON and SUVA. The variance in FI is high between sites, however, it provides the general trend of autochthonous and allochthonous DOM in the Grand River. DOC/DON, a well-established indicator of DOM source, did not show a useful relationship with FI during the surveys (Fig. 3.15). Protein-like fluorescence/DOC provides a good indicator of fresh autochthonous DOM and its relationship with DON- $\delta N^{15}$ (Fig. 3.11) suggests the source of N for autochthonous DOM may be different than allochthonous DOM.

The ranges for DOC/DON, FI, SUVA, MW and DON- $\delta N^{15}$ all suggest that DOM in the Grand River has large autochthonous contributions (Table 3.1). The downstream trend of these indicators shows that DOM in the Grand River increases in autochthonous sources from the headwaters to the middle reaches and decreases slightly in the lower river.

## 3.5 Discussion

### 3.5.1 Change in DOC and DON concentrations from headwaters to mouth

For all four seasons DOC concentrations decreased downstream and DON remained similar, peaking slightly in the middle reaches of the river. The largest variation in DOC concentration between seasons is in the headwaters.

Higher DOC concentrations upstream can be explained by input of water from flow through the upper soil zone due to low permeability of underlying till. Poorly drained soils have increased DOC concentration regardless of proportion of agricultural (Wilson and Xenopoulos 2008).

The decrease in DOC concentration from the headwaters is likely due to the contribution of four factors: processing in reservoirs, microbial/photodegration, land-use changes and changes in subsurface geology. Reservoirs are in the main channel of the Grand River (Lake Belwood) and Conestogo River (Lake Conestogo) before the middle reaches of the watershed. These reservoirs allow time for degradation to occur during some times of the year. Photodegradation readily breaks down allochthonous DOM (Amado et al. 2006) which is contributed by the upper portion of the watershed. The reservoirs allow more exposure time for this to occur. Additionally, the middle reach is made up of suburban catchments which contribute water with lower DOC concentrations due to removal of vegetation and upper soil horizons, ditching and retention ponds (Wahl et al. 1996). The lower DOC load seen in historical data in chapter 2 shows this reduction in the middle reaches. The lower-central portion further reduces the DOC concentration due to subsurface glacial outwash features in the area (Karrow and Morgan 2004). Groundwater recharge in sulphate containing sediments (Noseck et al. 2009) and well drained soils (Wilson and Xenopoulos 2008) contribute to a lower DOC concentration inputs and dilution.

DOC concentrations do not increase in the lower river despite the catchment being poorly drained and agricultural. This section has a high flow and smaller catchment area and may act more like a pipe carrying a large integrated signal. The lower Mississippi River, similarly, had little variation in DOC concentration (Duan et al. 2007b). The lower Hudson River showed the lowest DOC consumption and bacterial respiration compared with other portions of the river (del

Giorgio and Pace 2008). The lack of variation of DOC concentration in the lower Grand River can be explained by large influences from upstream.

DON concentrations increased slightly in the middle of the watershed. The suburban/urban land use and STPs in the middle reaches increase DON due to primary production of autochthonous DOM. The rest of the river showed little variation in DON concentration. This is due to quick processing of autochthonous DOM degraded mainly by bacterial mineralization (Amado et al. 2006).The most labile fraction of DOM is consumed very quickly (Kaplan et al. 2008). Middle section of river shows the most eutrophication (Rott et al. 1998) and the production rate is likely higher than degradation in this section.

### 3.5.2 Characterisations of DOM

DOC can show very little change when other characteristics of DOM can change significantly (Borisover et al. 2009). Characteristics like UV absorbance (SUVA and a250/a365), fluorescence (FI, protein-like, humic-like and P/HFlu), size analysis and stable isotopic ($\delta^{13}$C, $\delta^{15}$N) can provide insight into the sources and processes within the DOM pool. In complex systems like a large river many different sources of both allochthonous and autochthonous DOM affect these characteristics.

According to the river continuum concept, the headwaters will be dominated by allochthonous organic matter, the middle reaches will be dominated by autochthonous DOM with some residual refractory allochthonous organic matter and the lower river will have a gradual return to a higher proportion of allochthonous organic matter (Vannote et al. 1980).

In the headwaters (first 50km) of the Grand River the characteristics of DOM are more allochthonous than the rest of the river. A lower FI, protein-like fluorescence, P/HFlu and DON-$\delta^{15}$N and higher DOC/DON, SUVA shows that a larger portion of DOM is from an allochthonous source than the rest of the river. These inputs originate from runoff from poorly drained soils in small streams in the headwaters. Following this section of river (50 to 100km), an increase in autochthonous DOM is seen. This increase can be attributed to primary production in a wider channel and in the Lake Belwood reservoir. Consumption and degradation of semi-labile allochthonous DOM is occurring as it reaches a larger more productive river, accounting for the general trend of lowering of allochthonous indicators with downstream distance throughout the seasons.

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Urban land use and STP input have a strong influence on DOM characteristics. Autochthonous indicators show an increase in this section (100 to 165km). Less input of allochthonous DOM occurs in urban catchments (Wahl et al. 1996). Increased nutrient loading from STPs stimulates production of autochthonous DOM in this portion.

In the lower section of the river (165 to Lake Erie) autochthonous indicators decreased slightly. Nutrients decrease in this section and turbidity increases reducing primary production. However, DOC/DON and DON- $\delta N^{15}$ show that autochthonous DOM persists in the lower Grand River. Processes in the lower Grand River may be similar to those in the lower Hudson River which shows little bacterial respiration and may act more like a pipe than a reactor (del Giorgio and Pace 2008).

The total amount of DOM decreases downstream in the Grand River and remains constant in the lower river. The proportion of autochthonous DOM increases downstream while the proportion of allochthonous DOM decreases. Dilution from infiltration of groundwater is responsible for the overall reduction in DOM in the middle reaches. This dilution offsets the autochthonous production from increasing the overall DOM amount. Allochthonous DOM produced in the headwaters is also lost by UV photodegradation and microbial degradation.

June, September and October surveys showed little difference in DOM characteristics except in humic-like fluorescence/DOC which was higher in September. The February survey was higher in allochthonous indicators and lower in autochthonous indicators than the other surveys. Discharge during the February survey was the highest of the surveys. In the February survey, cold temperatures and ice cover limit degradation rates. Less degradation and low autochthonous production cause DOM in the Grand River to be predominately allochthonous in winter.

Generally the Grand River follows the river continuum concept. DOM is comparatively more allochthonous in the headwaters increasingly autochthonous in the middle reaches and decreases in autochthonous characteristics in the lower river. Although DOM in the headwaters is more allochthonous than the rest of the river, compared with literature values of exclusively terrestrial sources the DOM in general is more autochthonous. The headwaters of the Grand River are a greater source of autochthonous DOM than unimpacted model headwaters because of lack of shading and excess nutrients. The middle reaches of the river has extensive autochthonous production as expected by the river continuum concept. Human impacts in the

Grand River increase this production beyond that of a model unimpacted river but groundwater inputs lessen these effects. Based on the river continuum concept, the lower Grand River would be expected to increase in allochthonous DOM. This allochthonous increase (autochthonous decreases) in the lower Grand River is occurring at a lesser extent than if the river was a higher stream order (greater than 6). The Grand River follows the downstream trend of the river continuum concept but has a much higher autochthonous baseline due to human impacts.

### 3.5.3 Importance of Allochthonous and Autochthonous DOM in a large impacted river for management

DOM in large impacted rivers is a complex mixture of allochthonous and autochthonous DOM. Human impacts such as agriculture and urbanization result in a higher proportion of autochthonous DOM. Agricultural land use has been shown to increase autochthonous DOM in rivers (Wilson and Xenopoulos 2009). Nutrients released by STPs in the middle reaches of the river stimulate excessive macrophyte growth during the growing season (Hood 2011). Macrophytes are largely influenced by nutrient enrichment and water levels which are impacted by human activities (Hudon et al. 2005, Hudon and Carignan 2008). Macrophytes excrete labile autochthonous DOM (Mann and Wetzel 1996). Macrophytes have been shown to be a net source of autochthonous protein-like DOM (Lapierre and Frenette 2009). This labile DOM is readily consumed by microorganisms leading to oxygen depletion, a threat to ecosystem health. Autochthonous DOM has a higher disinfection byproduct forming potential, a concern to drinking water in downstream communities with the river as a water source (Jack et al. 2002).

Best management practices may lessen the impacts on DOM in the watershed. Use of riparian buffer zones with shading may help attenuate nutrients and lessen primary production in the headwaters. Optimal application of fertilizer to agricultural fields will avoid excess runoff and infiltration of nutrients into the tributaries of the Grand River. Upgrades to STPs to remove nutrients would lower autochthonous production in the middle reaches of the river. Implementation of these nutrient controls may be difficult due to socioeconomic factors. The population in the watershed is expected to increase by 50% in the next 20 years (Ministry of Public Infrastructure Renewal 2006). This population increase will contribute more nutrients and autochthonous DOM in the Grand River. Both the increased nutrient load and the increased demand for water from the Grand River will put more stress on the river ecosystem.

# Chapter 4: Assessing Indictors of Autochthonous DOM Contribution in Two Rivers of Contrasting Trophic Status

Summary

Dissolved organic matter (DOM) characteristics in two large rivers in Southern Ontario, Canada with differing anthropogenic impact were investigated. Downstream surveys of the agriculture intensive Grand River (population 900 000) and less developed Burnt River (population 20 000) were characterized by DOC concentration, DOC/DON, visual and UV absorbance (365nm, 254nm and 250nm), fluorescence index (FI), protein-like and humic-like fluorescence, and size exclusion chromatography (SEC). DOC/DON was lower in the Grand River than the Burnt River. FI and protein-like fluorescence normalized to DOC concentration were higher in the Grand River than the Burnt River. DOM in the Grand River generally had more autochthonous characteristics than the Burnt River likely due to inorganic nutrient loads stimulating primary production. The Burnt River showed little seasonal difference in DOM properties in contrast to the Grand River which was more autochthonous in the summer, allochthonous in the fall and a mixture in the spring. The anthropogenic impact on the Grand River has a strong effect on the DOM properties within the watershed compared to the less impacted Burnt River.

## 4.1 Introduction

DOM is one of the largest cycled organic carbon pools on Earth and the most bioavailable pool within the aquatic system. DOM can contain 1000s of different compounds making investigation of its characteristics difficult. Little study has been done to assess anthropogenic impacts on DOM characteristics. A recent study of 34 watersheds in Southern Ontario showed that, across a gradient of agricultural land use, the amount of autochthonous DOM increased as the cropland to wetland ratio increased based on fluorescence (Wilson and Xenopoulos 2009). This chapter will compare the DOM characteristics in the agriculture intensive Grand River (Fig. 4.3) and the less developed Burnt River (Fig. 4.1) watersheds.

In Chapter 2 it was shown that DOC concentration and DOC/DON varied seasonally, between the two extremes of summer/fall and winter/spring for seven years within the Grand River. Spring, summer and fall surveys were done to capture the full range of variability within

the Grand River and Burnt River.  This chapter uses several techniques to characterize DOM at the watershed scale. Visual and UV absorbance, 3D fluorescence, and size analysis characterization were done during these surveys to determine the source and biogeochemical processes affecting DOM. Large rivers have high variability in discharge and nutrient loading temporally and spatially that affect DOM characteristics. By surveying the entire length of these rivers seasonally this chapter captures the temporal and spatial changes of DOM in the river.

3D fluorescence allows construction of three-dimensional emission-excitation matrices (EEMs) which have recently become a valuable tool in characterization of DOM. EEMs can help in the understanding of DOM source and alteration/processing. Recent studies have shown EEMs to be useful in determining the influence of agricultural activities and waste water on DOM (Baker et al. 2004, Wilson and Xenopoulos 2009). 3D fluorescence EEMs were used to determine humic-like and protein-like fluorescence in this chapter for comparison of the Burnt and Grand Rivers. Humic-like fluorescence is indicative of allochthonous DOM and protein-like fluorescence is associated with autochthonous DOM (Lapierre and Frenette 2009).

The Grand River is highly impacted from source to mouth. As a result, downstream changes in DOM are not large and DOM is largely of autochthonous origin. A less impacted river, the Burnt River, has been selected to compare results of DOM characterisitics. The main objectives of this study are to compare the following in a highly impacted and a much less impacted river: 1) downstream evolution of DOM characteristics, 2) seasonal changes in DOM, and 3) the use of measures of allochthonous and autochthonous indicators of DOM source.

## 4.2 Study Site and Methods

### 4.2.1 Study Site

The two watersheds have similar discharge per area, $0.38 \times 10^6 \, m^3 \, year^{-1} \, km^{-2}$ for the Grand River and $0.42 \times 10^6 \, m^3 \, year^{-1} \, km^{-2}$ in the Burnt River. The amount of anthropogenic impact is different between the two watersheds; just under 1 million people live in the Grand River watershed compared to less than 20 000 permanent residents in the Burnt River watershed. Averages of the province water quality monitoring network (PWQMN) phosphorous data for the past eight years in the central portions of both rivers reflect the difference in trophic status. The Grand River had an average total phosphorous (TP) of 0.146 mg $L^{-1}$ and an average soluble reactive phosphorous (SRP) of 0.0480 mg $L^{-1}$ making it eutrophic. The Burnt River had an

average TP of 0.0168 mg L$^{-1}$ and an SRP of 0.0008 mg L$^{-1}$ making it oligotrophic to mesotrophic.

The Burnt River watershed is underlain with carbonate metasedimentary bedrock. This results in a higher pH than many other central Ontario waterways (Quinlan et al. 2003). The Grand River watershed is influenced by limestone and dolostone bedrock (Nelson et al. 2004). These carbonate rocks buffer the pH in the watersheds giving them similar water chemistries.

The headwaters of the Burnt River watershed are heavily populated by lakes (Fig. 4.2). This contrasts with the Grand River watershed that only has three sizable reservoirs: Luther Marsh, Belwood Lake and Conestogo Lake. Upstream lakes have been shown to affect the DOC concentration and properties. Autochthonous production, photodegradation and microbial processing in lakes change DOM properties (Larson et al. 2007a, Larson et al. 2007b). The different proportion of lakes in the Burnt River compared to the Grand River may have a strong effect on DOM properties.

The Grand River can be divided into three sections: The upper Grand River (sites 2 km to 97 km), central Grand River (sites 119 km to 187 km) and lower Grand River (sites 216 km to 295km). The upper Grand River is predominately agricultural land; the central Grand River has agriculture as well as intensive urbanization and sewage treatment plant (STP) inputs. The lower Grand River is underlain by a clay plain resulting in high turbidity. A study by Rott et al (1998) in the 1990's used diatom analysis to show that all three reaches had moderate to high pollution and moderate to high trophic level. The central Grand River had higher organic pollution and trophic level than the upper and lower Grand River (Rott et al. 1998). In terms of watershed size, the upper Grand River and the Burnt river watershed are comparable.

### 4.2.2 Methods

See Chapter 3

### 4.3 Results

### 4.4.1 DOC concentrations and DOC/DON ratio

DOC concentrations are generally similar in the Grand River and the Burnt River ranging from 3.8 to 19.6 mg l$^{-}$ (Fig. 4.4). DOC is higher in the headwaters of the Grand River (range 6.3 to 16.9 mg l$^{-}$, median 10.8 mg l$^{-}$) than in the Burnt River (range 3.8 mg l$^{-}$ to 19.6 mg l$^{-}$, median 7.6 mg l$^{-}$) for all seasons except for fall. DOC concentrations in the first 20 km of the Burnt

River are low compared with the downstream portion. The Drag River flows into the Burnt River at 40 km from the headwaters and a slight decrease in DOC concentration (by 0.8 to 2.2 mg l⁻) after the inflow was seen. In the Grand River, DOC decreased with downstream distance (r = -0.979, p = 0.000, n = 58) and the Burnt River DOC showed no downstream trend (p = 1.000, n = 35).

DOC concentrations were higher in the fall (mean 12.6 mg l⁻) season than the spring (mean 6.5 mg l⁻) or summer (mean 7.1 mg l⁻) (p = 0.000, p = 0.000) in the Burnt River. Conversely, Grand River DOC concentrations were significantly higher in the summer than the spring or fall (two-sample t-tests, p = 0.006, p = 0.014) for the length of the river.

The DOC/DON ratio was much lower in the Grand River (mean 15.6) than the Burnt River (mean 33.1) for the three surveys. The Grand River showed a decrease in DOC/DON with distance downstream (r = -0.488, p = 0.009, n = 58) while the Burnt River showed no trend (p = 1.000, n = 35). Fall DOC/DON in the Grand River was higher than spring or summer (two-sample t-tests, p = 0.000, p = 0.014). Burnt River spring and summer DOC/DON were not significantly different (two-sample t-test, p = 0.496) but both were significantly higher than summer (two-sample t-tests, p = 0.000, p = 0.000).

### 4.4.2 Nitrogen Concentrations

Figure 4.2 shows nitrogen species for surveys in the Grand and Burnt Rivers. TDN was higher in the Grand River compared with the Burnt River (p = 0.000). Dissolved inorganic nitrogen species (DIN), $NO_3^-$ and $NH_4^+$, were also significantly higher in the Grand River than the Burnt River (two-sample t-tests, p = 0.000, p = 0.000). Similarly, DON concentrations were lower in the Burnt River than the Grand River (two-sample t-test, p = 0.000).

In the Grand River, $NO_3^-$ is the largest species contributing to TDN. $NO_3^-$ shows a moderate positive correlation with downstream distance (r = 0.577, p = 0.000, n = 58) while $NH_4^+$ and DON do not show any trend (p = 1.000, n = 58). All the Grand River surveys showed an increase in DIN in the middle reach followed by a decrease in the lower river. In the Burnt River there was no downstream trend in nitrogen species (p = 1.000, n = 35). In the Grand River as $NO_3^-$ decreases DOC/DON increases, in the Burnt River $NO_3^-$ is lower and DOC/DON is higher than the Grand River (fig. 4.10).

Seasonal differences in the nitrogen in the Burnt River could not be seen. The Grand River showed slight differences in $NO_3^-$, highest in the spring and lowest in the fall.

### 4.4.3 SUVA and FI

SUVA provides an estimate of aromaticity. Higher aromaticity is characteristic of terrestrial DOM (Duan et al. 2007a). Cyanobacteria extract, a pure autochthonous DOM, had a low SUVA (1 L mg⁻) and Suwannee River humic acid, a predominately allothchonous DOM, had a high SUVA (7.4 L mg⁻) (Her et al. 2004). SUVA in the Grand River and the Burnt River ranged from 1.0 to 4.6 L mg⁻ and were not significantly different (two-sample t-test, $p = 0.374$) for the three surveys (Fig. 4.6). In the Grand River, SUVA is highest in the spring (two-sample t-tests, $p = 0.046$, $p = 0.007$) and is negatively correlated with downstream distance ($r = -0.656$, $p = 0.000$, $n = 58$). SUVA in the Burnt River is similar in the spring and summer (two-sample t-test, $p = 0.233$) but lower in the fall (two-sample t-tests, $p = 0.000$, $p = 0.048$). SUVA has no correlation with distance downstream the Burnt River.

FI ranged from 1.0 to 2.0 in the Grand and Burnt Rivers and was higher in the Grand River than the Burnt River (two-sample t-test, $p = 0.004$). FI was lower in the headwaters in the Burnt River in the spring and fall but showed no correlation with downstream distance ($p = 1.0$). In the Grand River, FI was higher in the middle river and showed no correlation with downstream distance ($p = 1.0$). FI was not significantly different between seasons in the Burnt River and was significantly higher in than fall than the spring in the Grand River (two-sample t-test, $p = 0.038$).

### 4.4.4 Molecular weight and size

Absorbance at 250nm/365nm (a250/a365) provides an estimate of low-molecular-weight to high-molecular-weight DOM (Fig. 4.7). a250/a365 is higher in the Burnt River than the Grand River for the three sets of surveys (two-sample t-test, $p = 0.002$). Peak apparent molecular weight (MW) determined by size exclusion chromatography provides an estimate of average molecular size. MW is lower in the Burnt River than the Grand River (two-sample t-test, $p = 0.014$) for all surveys. Both absorbance 250/365 and MW indicate the size of the DOM in the Grand River contains generally larger DOM than the Burnt River. In the Grand River the summer was lower than spring and fall for MW (two-sample t-tests, $p = 0.000$, $p = 0.000$) and a250/a365 (two-sample t-tests, $p = 0.022$, $p = 0.000$). Similarly, the MW was lower in the summer than the fall (two-sample t-test, $p = 0.009$) in the Burnt River and a250/a365 was also higher in the summer than spring and fall (two-sample t-tests, $p = 0.033$, $p = 0.009$).

### 4.4.5 Humic-like and Protein-like Fluorescence

Both humic-like and protein-like fluorescence normalized to DOC concentration were higher in the Grand River than the Burnt River (two-sample t-tests, p = 0.000, p = 0.000) (Fig. 4.8). During the Burnt River surveys humic-like and protein-like fluorescence normalized to DOC were negatively correlated to DOC (r = -0.692, p = 0.000, n = 35),( r = -0.731, p = 0.000, n = 35) and DOC/DON (r = -0.610, p = 0.008, n = 35),( r = -0.720, p = 0.000, n = 35). Humic-like fluorescence normalized to DOC was not correlated to any other parameters in the Grand River surveys. Normalized protein-like fluorescence in the Grand River, however, was negatively correlated to DOC (r = -0.631, p = 0.000, n = 58) and DOC/DON (r = -0.599, p = 0.000, n = 58) and positively correlated to TDN (r = 0.752, p = 0.000, n = 58), $NO_3^-$ (r = 0.720, p = 0.000, n = 58), $NH_4^-$ (r = 0.568, p = 0.000, n = 58), and downstream distance (r = 0.639, p = 0.000, n = 58).

The ratio of protein-like fluorescence to humic-like fluorescence (P/HFlu) may provide a useful parameter for the proportion of autochthonous to allochthonous DOM (Fig. 3.7). P/HFlu is negatively correlated to DOC (r = -0.541, p = 0.001, n = 82) and DOC/DON (r = -0.634, p = 0.000, n = 82) and positively correlated to TDN (r = 0.700, p = 0.000, n = 58), $NO_3^-$ (r = 0.642, p = 0.000, n = 58), $NH_4^-$ (r = 0.574, p = 0.000, n = 58), and downstream distance (r = 0.546, p = 0.001, n = 58) in the Grand River. P/HFlu in the Burnt River was not correlated to any other parameters and was higher than in the Grand River (two-sample t-test, p = 0.000).

Normalized humic-like fluorescence was higher in the spring than summer and fall (two-sample t-tests, p = 0.000, p = 0.000) and normalized protein-like fluorescence was lower in the fall than spring and summer (two-sample t-tests, p = 0.000, p = 0.000) in the Grand River. In the Burnt River, normalized humic-like fluorescence was lower in the fall than the spring and summer (two-sample t-tests, p = 0.000, p = 0.000) and normalized protein-like fluorescence was higher in the summer than spring and fall (two-sample t-tests, p = 0.001, p = 0.001).

### 4.4 Discussion

### 4.4.1 Sources and Sinks of DOM in the Grand and Burnt watersheds

The source of DOM in the Grand River watershed is from agricultural and wetland soils as well as primary production within the river channel. In the Burnt River watershed the source of DOM is from forest soils and wetlands. It would be expected that the Grand River DOM would be of higher autochthonous character while the Burnt River DOM would be of

allochthonous character based on these sources. Sinks in the Grand River include photodegradation, microbial degradation in the river channel and reservoirs. In the Burnt River sinks are similar, however, the watershed contains more lakes allowing more time for photodegradation. The poorly drained agricultural soils in the headwaters of the Grand River watershed result in a high DOC concentration. Alternatively, in the Burnt River, processing in lakes of headwaters results in lower DOC concentrations.

## 4.4.2 Measures of allochthonous and autochthonous as indicators of DOM source

Table 4.1 summarizes indictors of DOM in the Burnt River and upper, central and lower Grand River during summer surveys. The central Grand River has the highest trophic status of the three river sections (Rott et al. 1998). The most autochthonous DOM in the waters studied is expected in summer in the central Grand River. The central Grand River had low DOC/DON, SUVA, protein-like fluorescence/humic-like fluorescence and elevated FI and protein-like fluorescence/DOC compared to the upper Grand River and Burnt River. These properties are potentially useful in discrimination between autochthonous and allochthonous DOM.

The high proportion of lakes in the Burnt River watershed may have a strong effect on DOM indicators independent of source. DOC concentration and SUVA (Kelton et al. 2007, Larson et al. 2007a) as well as MW and fluorescence properties (Mostofa et al. 2007, Opsahl and Benner 1998) are decreased by photodegradation and processing. Increased residence time in lakes exposes DOM to more photodegradation than in a predominantly riverine system. This in-lake processing may be causing the lower apparent MW, SUVA, and DOC concentration compared to the upper Grand River. DON is more resistant to photodegradation than DOC and photodegradation will decrease DOC:DON (Buffam and McGlathery 2003). Photodegradation makes discerning autochthonous versus allochthonous DOM more difficult since photodegradation shifts many DOM properties towards the apparent autochthonous direction.

## 4.4.3 Are the Grand and Burnt Rivers primarily allochthonous or autochthonous?

Burnt River DOM is affected by the watershed's many lakes. Nutrient loading is low in the watershed which is oligotrophic to mesotrophic meaning primary productivity is relatively low. DOM in the watershed is expected to be allochthonous although several properties are out of the literature range of an allochthonous source. Apparent MW, humic-like fluorescence/DOC, protein-like fluorescence/humic-like fluorescence and SUVA of DOM in the Burnt River are

lower than would be expected for allochthonous sources. This may be due to in-lake processing and photodegradation. Numerous studies have shown solar irradiance lowers the MW of DOM (Bertilsson et al. 1999, Kohler et al. 2002, Opsahl and Benner 1998, Osburn et al. 2001). This is consistent with the apparent MW of Burnt River DOM which is significantly lower than the apparent MW in the Grand River. A study by Mostofa et al (2007) investigated the effects of irradiance on fluorescence peaks similar to humic-like fluorescence and protein-like fluorescence used in this study. Protein-like fluorescence was shown to be less susceptible to photodegradation than humic-like fluorescence (Mostofa et al. 2007). Humic-like fluorescence/DOC was likely lower in the Burnt River than the Grand River due to increased photodegradation. Upstream lakes in river watersheds cause the alteration of terrestrial DOM making prediction of DOM properties difficult (Larson et al. 2007b). Similarly, protein-like fluorescence/humic-like fluorescence was likely higher in the Burnt River due to the differential photodegradation of humic-like fluorescence over protein-like fluorescence. SUVA, a measure of UV absorbance was significantly lower in the Burnt River than the Grand River. It has been shown that exposure to solar radiation causes a reduction of UV absorbance in DOM (Osburn et al. 2001). The DOM properties in the Burnt River that are out of the range expected for allochthonous sources can be explained by photodegradation.

The Grand River DOM was generally more autochthonous than the Burnt River. DOC/DON and FI are well established indicators of autochthonous versus allochthonous DOM (McKnight et al., 2001, Hood et al., 2005). During the surveys, DOC/DON was lower and FI was higher in the Grand River compared to the Burnt River. Autochthonous DOM contains more protein-like components (Elfrida et al., 2009), the protein-like fluorescence/DOC concentration was consistently higher in the Grand River than in the Burnt River. DON and protein-like fluorescence are more resistant to photodegradation than many other indicators (Buffam and McGlathery 2003, Mostofa et al. 2007, Wiegner and Seitzinger 2001). Therefore, DOC/DON and protein-like fluorescence may be ideal for comparing sources in watershed with differing exposure to photodegradation.

### 4.4.4 Downstream evolution of DOM characteristics

The less impacted Burnt River showed no significant change in DOM characteristics downstream in contrast to the impacted Grand River. The Grand River showed a decrease in DOC concentrations, DOC/DON ratio, and SUVA downstream and an increase in $NO_3^-$,

normalized protein-like fluorescence and protein-like fluorescence/humic-like fluorescence downstream. These downstream changes in the Grand River indicate DOM is becoming more autochthonous downstream likely due to the impact of the Waterloo and Kitchener STPs at 119 km and 140 km, respectively.

## 4.4.5 Seasonal changes in DOM

The Burnt River and Grand River showed different seasonal trends in DOM characteristics. The Grand River generally showed higher DOC and DON concentrations in the summer compared to the fall. DOM in the fall in the Grand River showed indications of being more allochthonous in character than the summer with a larger size, higher DOC/DON ratio and lower protein content. DOM in the spring and summer were similar in many autochthonous indicators including DOC/DON ratio, FI, and protein content but the spring DOM was larger and was more aromatic and humic based on size estimates, SUVA and humic-like fluorescence. DOM in the Grand River was mainly of autochthonous origin in the summer survey, allochthonous in the fall and a mixture of autochthonous and allochthonous in the spring. The spring survey was in the late spring likely contributing to a higher proportion of autochthonous DOM due to increased production.

The Burnt River showed less seasonal trends in DOM properties. No significant seasonal trends were seen in autochthonous indicators FI and protein-like fluorescence/humic-like fluorescence but summer had a smaller size, lower DOC/DON ratio and higher protein-like fluorescence/DOC. Fall had high DOC concentrations but low SUVA and humic-like fluorescence/DOC. The summer survey had the highest proportion of autochthonous DOM, but was lower than any Grand River survey. The fall survey had the lowest optical allochthonous indictors which may have been due to photobleaching in lakes.

## 4.5 Conclusions

Human activities in the Grand River are having an impact on the DOM characteristics in the Grand River watershed compared to the Burnt River watershed. The cumulative impacts of human activities can be seen downstream in the Grand River while the Burnt River is more homogenous. Grand River DOM has a greater seasonal cycle than the Burnt River because of high primary production in warmer months due to inorganic nutrients. A high proportion of lakes in the Burnt River allows for more photodegradation of DOM. Photodegradation causes

significant changes in DOM properties making discrimination of autochthonous and allochthonous sources more difficult, however, the difference between the Burnt River and the Grand River is clear. Protein-like fluorescence/DOC concentration is a good indicator despite photodegradation. DOM from primary production in the Grand River contributes to a higher protein and organic nitrogen component. This type of DOM is different than the DOM in the Burnt River and therefore has a different ecosystem function. Since DOM in the Grand River is more bioavailable this can contribute to oxygen depletion during consumption which is a threat to ecosystem health.

# Chapter 5: Organic Matter and Disinfection By-products in the Grand River Watershed

Summary

During the disinfection of drinking water, chlorine reacts with dissolved organic matter (DOM) to produce potentially harmful disinfection byproducts (DBPs). Trihalomethanes (THMs) and haloacetic acids (HAAs) are major types of DBPs and have been shown to be toxic. In the Grand River watershed, chlorination of surface water for drinking is potentially harmful to human health. The communities of Ohsweken and Brantford get 100% of their drinking water from the Grand River. Drinking water surveillance program (DWSP) data from 1998 to 2004 was analyzed to investigate potential risks. After treatment and chlorination, levels of THMs were $\geq 50 \mu g/l$; a level shown to increase the risk of various types of cancer when exposed over several decades. DBPs in drinking water are reported as an annual average in Ontario. However, seasonal changes are an important consideration. In the Grand River, the summer and fall seasons have higher DOM concentrations than late winter and spring, which peak in the late fall or early winter. DBPs may be higher during these seasonal peaks in DOM. Br$^-$ from STPs can react during treatment to form more toxic brominated halomethanes (BHMs) which are not currently tested for or regulated.

## 5.1 Introduction

Disinfection is the most important process in the reduction of pathogens in drinking water supplies. Chlorination has allowed water related infectious diseases from water supplies of the industrialized world to be virtually eliminated. However, the oxidizing strength of chlorine that destroys pathogens is also reactive with organic matter (OM) and produces potentially harmful disinfection byproducts (DBPs) (Kleiser and Frimmel 2000, Sirivedhin and Gray 2005).

### 5.1.1 Chlorinated Disinfection Byproducts

Chlorine gas ($Cl_2$) added to water quickly hydrolyzes into hypochlorous acid (HOCl). HOCl in turn reacts with dissolved organic matter (DOM) to form over 300 types of DBPs (Environment Canada and Dept. of National Health and Welfare 1993). The main types of DBPs include trihalomethanes (THMs) and haloacetic acids (HAAs). HOCl also oxidizes bromide (Br$^-$)

into bromine, which is much more reactive with DOM than chlorine, and results in production of brominated halomethanes (BHMs) (Environment Canada and Dept. of National Health and Welfare 1993, Sirivedhin and Gray 2005). THMs, HAAs and BHMs are known to be carcinogenic to humans, causing cancers in vital organs (Chow et al. 2007, Environment Canada and Dept. of National Health and Welfare 1993, Gopal et al. 2007, Zhang and Minear 2002). BHMs are more cytotoxic and mutagenic than chlorinated DBP (Chow et al. 2007, Environment Canada and Dept. of National Health and Welfare 1993, Gopal et al. 2007, Zhang and Minear 2002). Haloactonitriles (HANs) are toxic nitrogen-containing disinfection byproducts (N-DBPs). Alternative disinfection agents to chlorine such as chloramines may produce more N-DBPs and HANs than chloration alone (Hayhoe et al. 2007, Muellner et al. 2007). N-nitrosodimethylamine (NDMA) is a type of nitrosamine that is a N-DBP which has recently become a concern in drinking water supplies (Andrzejewski et al. 2005). Of further concern is the fact that dissolved organic nitrogen (DON) rich waters disinfected with chlorine or chloramines yield increased levels of NDMA and other N-DBPs (Dotson et al. 2009).

THMs are volatile substances that not bioavailable and do not partition into tissues or sediments. The half-life of THM is approximately 0.3-3 days in a river before being volatilized (Environment Canada and Dept. of National Health and Welfare 1993, Zoeteman et al. 1980). THMs should not therefore persist in the Grand River for any length of time. However, constant discharge of CWWE into the Grand River results in point source plumes that may exist for kilometers downstream (Murry 2008). CWWE have been deemed toxic by the Canadian CPA for the residual chlorine and chlorination DBPs (Environment Canada and Dept. of National Health and Welfare 1993).

### 5.1.2 Health Effects

In a study of chlorinated surface waters in Ontario, it was determined that the risk of bladder cancer was 1.63 times greater for those exposed to $\geq 50 \mu g/l$ of THM for more than 35 years compared to those exposed for less than 10 years (King and Marrett 1996). A recent study on the increased risk of adult leukemia found a 1.72 risk factor for an exposure of $\geq 40 \mu g/l$ of THM for more than 30 years (Kasim et al. 2006). Similar risk factors have been found for numerous other cancers (Backer et al. 2008).

The Ontario drinking water quality standard maximum for THM levels is an annual average of $100 \mu g/l$ (Ontario. Ministry of the Environment 2003), double what various

epidemiological studies used as their maximum cutoff group (King and Marrett 1996). In Ontario, no regulation exists for HAAs which is a health concern (Sirivedhin and Gray 2005). The United States EPA is more prudent when it comes to setting limits for levels of DBPs. The maximum EPA contaminant level for THMs is 80μg/l and 60μg/l for HAAs (Gopal et al. 2007).

NDMA is highly carcinogenic and mutagenic. Consequently, the US EPA has set a maximum level of 7ng/l of NDMA in drinking water (Andrzejewski et al. 2005). The maximum limit in Ontario is 9ng/l (Ontario. Ministry of the Environment 2003).

### 5.1.3 Organic Matter

OM originates from both autochthonous and allochthonous natural sources. Allochthonous sources include terrestrially derived materials in various degraded forms from soils in the riparian zone, and plant detritus (Jack et al. 2002). Autochthonous OM is produced within the aquatic environment by macrophytes, algae, and periphyton (Chow et al. 2007, Jack et al. 2002). Another potentially significant source is the release of OM in WWTP effluent (Sirivedhin and Gray 2005).

OM can occur as either dissolved (DOM) or particulate (POM) in aquatic systems. DOM is more reactive with chlorine and has the highest disinfection byproduct formation potential (DBP-FP) (Jack et al. 2002).  DOM can be quantified through elemental analysis of carbon (DOC) and nitrogen (DON). DBP-FP is higher in DOM with a higher DON content (Lee et al. 2007, Reckhow et al. 1990). DON is also a precursor to HANs and NDMA which are more toxic than carbon-based DBP (Andrzejewski et al. 2005, Muellner et al. 2007). DBP-FP is determined by quality and quantity of OM. The specific absorbance at 254nm divided by DOC concentration (SUVA) is a useful quantification of aromaticity (Weishaar et al. 2003). Higher DBP-FP is seen in DOM with higher aromatic content (Kitis et al. 2001, Reckhow et al. 1990).

Environmental factors influence DBP-FP within a watershed. DOM varies seasonally both in quantity and quality. Fall and spring flushing causes an influx of allochthonous DOM from terrestrial sources while late spring/early summer aquatic plant growth results in autochthonous DOM production. Allochthonous DOM is more aromatic with a higher DON/DOC ratio than autochthonous DOM (Hood et al. 2005, Jack et al. 2002). The high aromaticity of allochthonous DOM gives it a high DPB-FP. Alternatively, the high DON content of autochthonous DOM increases its DBP-FP as well. In headwater catchments, DOM is typically dominated by allochthonous material (Hood et al. 2005, Hood et al. 2005, Vrac et al.

2007) while in larger river sections autochthonous DOM can also be significant. In the Ohio River algal production was correlated to THM production, showing that algal production and senescence led to increased DPB-FP (Jack et al. 2002). WWTPs are another source of OM in the river environment. DBP-FP per DOM concentration of sewage effluents has been found to be lower than natural DOM (Sirivedhin and Gray 2005). However, effluents have much greater concentrations of DOM than occur naturally and thus will result in more DBPs formed even at low DBP-FP. Also, $Br^-$ concentration is higher in effluents causing greater formation of BHMs than natural DOM (Sirivedhin and Gray 2005).

The cities of Brantford and Ohsweken first nation village receive 100% of their drinking water directly from the Grand River (Ontario. Ministry of the Environment 2007, 2006, 2005, 2004, 2003). The drinking water intake for Brantford and Ohsweken are below all of the Region of Waterloo WWTPs. The Region of Waterloo also uses the Grand River as a source for 20% of its drinking water (Waterloo Regional Council 2000). OM from natural and anthropogenic sources in the Grand River is a possible concern due to the formation of DBPs during treatment of drinking water.

DBPs within the Grand River watershed in Ontario, Canada will be investigated. Two separate aspects of exposure to DBPs are of concern, CWWE released into the Grand River and drinking water taken from the Grand River. There are several major wastewater treatment plants (WWTPs) releasing CWWE into the Grand River, including the 14 WWTPs serving the Region of Waterloo and Brantford. Together these plants process wastewater from approximately 600 000 people (Ontario. Ministry of Public Infrastructure Renewal and Ontario. Ministry of Public Infrastructure Renewal 2006, Ontario. Ministry of the Environment. Hazardous Contaminants Coordination Branch and Ontario. Water Resources Branch ). The Region of Waterloo discharges 182 000 $m^3$/day of waste water into the Grand River and its tributaries (Waterloo Regional Council 2000). There is concern that CWWE DBPs may be having an effect on the Grand River ecosystem.

The objectives of this chapter are: 1) to access the risk of DBPs on human health in drinking water and ecosystem health in the Grand River watershed and the relationship to OM; 2) use Provincial water quality monitoring network (PWQMN) data (from chapter 2) and longitudinal surveys of the Grand River (from chapter 3) to establish seasonal and downstream trends of OM and the potential for DBPs formation.

## 5.2 Methods

Provincial water quality monitoring network (PWQMN) data was obtained from the Ontario Ministry of the Environment for the last four decades. The DOC concentration data during the 1980s provided the most complete data set and was therefore analyzed to provide insight into seasonal changes. For more information refer to chapter 2.

Concentrations of DBPs in treatment plants and distribution systems in the Grand River watershed were obtained from the Ontario Ministry of the Environment Drinking Water Surveillance Program (DWSP). THM, HAA and NDMA concentrations from 1998 to 2004 were averaged for the raw water, treatment plant and distribution system for Waterloo, Kitchener (at Mannhiem), Brantford, Ohsweken and Dunnville.

On June 26 2007, a survey of the entire Grand River was conducted, with samples collected at approximately every 20 km. Water chemistry and the quantity and quality of OM was analyzed for each site. $Br^-$ was run on a Dionex ion chromatograph. DOC, DON, TN, and $NH_4^+/NH_3$ was analyzed by the methods used in Chapter 3.

## 5.3 Results/Discussion

### 5.3.1 DBPs in drinking water systems in the Grand River watershed

The THM levels in several drinking water distribution systems in the Grand River watershed are shown in Table 5.1. The Region of Waterloo (Waterloo and Kitchener) distribution system is low in THMs since 80% of the water is from groundwater (Waterloo Regional Council 2000) which typically has a low DOM concentration. Brantford and Ohsweken drinking water THM concentrations are high since 100% of their water comes from the Grand River. Brantford and Ohsweken water is within the range of THMs that studies have shown to have an increased risk of certain types of cancers. THMs are low in Dunnville since its source is Lake Erie which is lower in DOM (mean DOC 2.4 mg l⁻, mean DON 0.2 mg l⁻ at drinking water intake) than the Grand River (mean DOC 5.2 mg l⁻, mean DON 0.7 mg l⁻ at drinking water intakes).

HAA levels show similar patterns in the distribution systems to THMs (Table 5.2). Brantford and Ohsweken levels are the highest and Waterloo is the lowest. The average levels at

Ohsweken exceed the US EPA maximum contaminant level of 60 μg l⁻ and the levels in Brantford are slightly lower.

Average NDMA concentrations are shown in Table 5.3. The Brantford distribution exceeds the US EPA maximum of 7ng l⁻ but is slightly under the Ontario maximum of 9 ng l⁻. The average NDMA levels in the Ohsweken distribution system were higher than those in Kitchener, Waterloo and Dunnville but did not exceed any limits.

Preliminary work has been done to understand the toxicity of lesser studied DBPs. BHMs have been shown to cause a greater chromosomal aberration than chlorinated DBPs (Echigo et al. 2004). Br⁻ in the Grand River ranges from 0 to 0.67 mg l⁻ and peaks below the major STPs, persisting downstream. In waters with a Br⁻/DOC ratio of 0.1, the BHMs may account for 29% of the toxicity of chlorinated DBPs (Echigo et al. 2004). Figure 5.1 shows Br⁻/DOC for a survey of the Grand River. The two sites before the Brantford intake have Br⁻/DOC ratios of 0.11 and 0.090, respectively. This would suggest many of the DBPs formed during treatment in Brantford could be BHMs which are not tested for, or regulated in Ontario (Ontario. Ministry of the Environment 2003).

N-DBPs have been shown to be more toxic than carbon containing DBPs in cell assays (Muellner et al. 2007). The DOC/DON ratio is important in determining how much N-DBPs are formed. As the DOC/DON decreases, the amount of N-DBPs formed increases when adding free chlorine (Muellner et al. 2007). Figure 3.2 shows a decrease in the DOC/DON ratio below the large sewage treatment plants, likely due to an increase in autochthonous production. However, the DOC/DON ratio recovers slightly before the Brantford water intake.

Drinking water systems in the Grand River watershed supplied solely from the river have DBP levels in the range of a risk to human health. Few long term studies have been done on DBPs in drinking water and health. Levels present in Brantford and Ohswegen exceed 50μg/l THMs, a level shown to increase cancer risk under long term exposure (<35 years) (Backer et al. 2008, Kasim et al. 2006, King and Marrett 1996). High Br⁻ and DON in the Grand River at the intakes of Brantford and Ohswegen may cause production of BHMs, HANs and NDMA during chlorination of drinking water. BHMs and HANs are not measured, but NDMA levels are elevated in distribution systems of Brantford and Ohswegen. BHMs, HANs and NDMA are more toxic than THMs (Andrzejewski et al. 2005, Gopal et al. 2007, Muellner et al. 2007) and

OM and conditions are optimal for formation in drinking waters within the Grand River watershed presenting a risk to human health.

The effect of DBPs on ecosystem health and aquatic organisms is not well understood. The Waterloo and Kitchener, CWWEs are been detected undiluted as far as 10km downstream of the outfall using conductivity measurements (Murry 2008). Water quality below the Kitchener is poor due to the influence of the CWWEs (Nelson et al. 2004). However, there is a complex mixture of inorganic nutrients, residual chlorine and other trace pollutants that may be causing this. Mortality of caged juvenile rainbow trout 100 meters below the Waterloo WWTPs was 100% while control whole effluents that had not been chlorinated showed no mortality (Environment Canada and Dept. of National Health and Welfare 1993). Benthic communities exposed to chlorinated effluents showed a decrease in species richness and favoured a single taxa tolerant to pollution. Non-chlorinated effluents showed a greater species richness in the benthic community. Due to the complex mixture of the CWWEs it has not been possible to determine whether residual chlorine or DBPs cause the change in community structure (Environment Canada and Dept. of National Health and Welfare 1993).

OM matter in the source water is directly related to DBPs formed in drinking water. This relationship can be seen in DBP levels in Brantford, Ohsweken, Kitchener, Waterloo and Dunnville. Source water in Waterloo is groundwater and has very low OM compared to the surface water sources used by Brantford, Ohsweken and Dunnville. OM in Lake Erie is lower than the Grand River, the mean DOC concentration in Lake Erie in 1997 was 2.7 mg/l (Smith et al. 1999). Since the source water for Dunnville is from Lake Erie, which is low in OM, DBPs in drinking water is lower compared to Brantford and Ohsweken where the drinking water source is the Grand River, a source higher in OM.

Historical PWQMN data from 1981 to 1987 (Table 2.3) has a record of two sites (Glen Morris and York) near the Brantford and Ohsweken intakes. Mean DOC concentrations were 5.5 and 5.1 mg/l and mean DON concentrations were 0.75 and 0.74 mg/l for the seven year period at Glen Morris and York. DOC concentration in the spring and winter was lower (~5 mg/l) than the summer and fall (~6 mg/l). DON concentrations were lowest in the winter (0.65 mg/l) followed by the spring (0.74 mg/l) and summer (0.78 mg/l) and fall (0.83 mg/l). The drinking water source for Brantford and Ohsweken are consistently high in OM and nitrogen content. This shows there is a consistent risk of DBP formation in these water systems. Seasonally there is high OM and

lower C:N in summer and fall likely due to increased primary production. Greater formation of DBPs is likely during the summer and fall and formation of more toxic N-DBPs such as HANs and NDMA.

Sampling surveys during 2007 and 2008 (Figure 3.2, 3.3, 4.4, 4.5) showed similar trends to historical data at the corresponding sites: 164 km (Glen Morris) and 253 km (York). Mean DOC concentrations for the two year period were 6.6 mg/l at Glen Morris and 5.8 mg/l at York and mean DON concentrations were 0.77 mg/l and 0.56 mg/l. This shows that OM decreases due to downstream processing and that the higher levels of DBPs at Ohsweken compared to Brantford is likely due to less treatment for OM at Ohsweken.

## 5.4 Implications

Removal of DOM during treatment would eliminate the majority of DBPs. The goal of removal of DOM may be possible as treatment for drinking water. However, this may require extensive upgrades to wastewater treatment plants. UV irradiation and ozonaton serve as alternative disinfection methods. However, chlorination is usually still required after treatment to stop pathogens from growing in the distribution system. Ozonation byproducts have not been studied for toxicity and it is not clear if they are harmless (Joss et al. 2008). Other emerging treatment processes to remove DOM such as ion exchange may help reduce DBPs (Tan et al. 2005). The use of chloramines, instead of chlorine, reduces the DBPs formed during treatment but more is required to disinfect. Chloramines do not stay in the water long enough to pass through the distribution system. Chloramines form 10 times less THMs but 5 times more HANs which may be more toxic (Lee et al. 2007).

Better management of the watershed may help reduce the load of OM. A reduction in inorganic nutrients would reduce the OM associated with aquatic primary production in the spring and summer. Better management practices such as creating riparian buffer zones could reduce these nutrients as well as reduce erosion leading to high OM loads in the fall.

Because of the high OM content of sewage effluent, alternative methods of disinfection may not be possible. Dechlorination is a solution that may be implemented in the future by many WWTPs but has a higher cost associated with it. Dechlorination may remove the residual chorine but may not remove the DBPs formed by the previous chlorination reaction. New technologies and upgrades in waste water treatment may help reduce DBPs, but the cost associated with these may be great. If possible effluents could not be chlorinated, avoiding many problems but

pathogens may present a problem downstream. Nitrification effluents are starting to be become a standard in treatment of effluents to remove the toxic effects of ammonia. In CWWEs containing no ammonia, DBP-FP is 5.3 times higher (Rebhun et al. 1997). Upgrading treatment processes to nitrify may cause other problems such as higher DBPs.

Chlorination is an essential disinfection process. Removal of pathogens should never be sacrificed for the removal of DBPs in drinking water. Pathogens cause acute illness to the general population, while DBPs cause chronic long term illness to a very small portion of the population. As more treatment technology is developed it may be possible to replace chlorination or remove OM before DBPs can form. Many areas, especially those with smaller population centres, such as Brantford and Ohsweken, cannot afford major treatment plant upgrades and may have an increased risk of some cancers.

The most sensible solution to reduction of DBPs is the protection of groundwater resources. Groundwater has a low organic content and groundwaters fed populations, like the Waterloo Region, have much lower quantities of DBPs associated with their treatment. The projected population growth in the Waterloo Region will require its reliance on a pipeline from Lake Erie which could increase its DBP content in drinking water (Waterloo Regional Council 2000).

The costly upgrade of WWTPs to remove OM before chlorination or the removal of the chlorination process all together may be the only options for reducing DBPs in effluents.

# Chapter 6: Conclusion and Future work

## 6.1 Summary of conclusions

Several objectives were achieved in this thesis to characterize DOM in the Grand River. The first objective was to describe long term seasonal and longitudinal trends in the Grand River using provincial water quality monitoring network data (PWQMN) data with monthly monitoring of seven sites from 1981 to 1988. Using DOC/DON as an indicator of the relative autochthonous to allochthonous contribution to DOM in the watershed it was shown that autochthonous DOM increased downstream and with increasing proportion of agricultural land. Seasonally, the DOM in the headwaters was more autochthonous in the spring and winter than the summer and fall. The lower-central river DOM was the most autochthonous in the summer and most allochthonous in the winter.

The second objective was to determine change in DOM properties in the Grand River Watershed seasonally and longitudinally at high resolution. Seasonally, Grand River DOM in the river as a whole was the most autochthonous in the late summer and the most allochthonous in the winter. DOM in the Grand River was shown to generally follow the river continuum concept, relatively allochthonous in the headwaters, most autochthonous in the middle reaches and plateauing in the lower river. Nutrients from large sewage treatment plants (STPs) in the central watershed increase productivity and make this section the most autochthonous. Protein content was strongly corrected to $\delta^{15}$N-DON in the Grand River. Both protein content and $\delta^{15}$N-DON show the effects of major STPs and are good indictors of autochthonous DOM.

The third objective was to compare measures of allochthonous and autochthonous indictors of DOM source by examining the change in DOM characteristics in an impacted versus a less impacted river. DOM properties in the agricultural and urban Grand River watershed and the less impacted Burnt River watershed were directly compared along the length of the river. The high proportion of lakes in the Burnt River watershed makes discrimination of autochthonous and allochthonous sources more difficult due to photodegradation. Photodegradation reduces allochthonous properties of UV absorbance, humic-like fluorescence and size. Despite photodegradation, DOC/DON and protein-like fluorescence/DOC, which are more resistant to photodegradation, show that the Grand River is more autochthonous than the Burnt River. The difference between the watersheds can be explained by anthropogenic land use

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increasing nutrient loading and stimulating primary production resulting in autochthonous DOM that is high in protein content and organic nitrogen.

The forth objective was to investigate the impact of autochthonous versus allochthonous DOM on ecosystem and human health by examining disinfection by-products (DBPs) in literature and the drinking water surveillance program (DWSP) within the Grand River watershed. Drinking waters in the watershed with more autochthonous DOM showed more DBPs and more toxic forms of DBPs. $Br^-$ inputs from STPs can react with DOM during water treatment to form more toxic forms of DBPs that are not currently analysed for or regulated. Drinking waters in the Grand River watershed show DBP levels in the range high enough to risk human health. The effect of DBPs on the aquatic ecosystem is not well understood due to the complex mixture of pollutants in effluent from STPs.

Inorganic nutrients have a great effect on aquatic ecosystem dynamics and DOM. Fig. 6.1 shows an inorganic nutrient, $NO_3^-$, and its relationship to DOC/DON in all sites in this thesis and from literature. $NO_3^-$ is chosen due to its prevalence in the literature, since N can be limiting, and if P is limiting nutrient pollution often contains both N and P inorganic pollutants. This relationship shows that in aquatic systems with higher nutrients, expressed as $NO_3^-$, will have autochthonous DOM. Low DOC/DON is seen above 0.5 mg l⁻ which may simply indicate nutrient enriched waters contain more autochthonous DOM. DOC/DON was lower in the 1980s than 2007-2008, this was due to higher DOC in 2007-2008 since DON between the two time periods was not significantly different.

Protein-like fluorescence has been shown to be an indicator of autochthonous DOM and humic-like fluorescence of allochthonous DOM (Lapierre and Frenette 2009). Additionally, DOC/DON has been a well-established indicator of DOM sources. Protein-like and humic-like fluorescence and DOC/DON were used as factors for autochthonous and allochthonous DOM in the conceptual graph shown in Fig. 6.2 combining all 2007 and 2008 Grand River surveys. This shows the downstream evolution in DOM shifting from a larger proportion of allochthonous DOM in the headwaters to autochthonous. Autochthonous DOM is greatest below the major STPs in the central proportion of the graph. The theoretical concentration of allochthonous DOM, however, decreases significantly. DOC concentration and allochthonous DOM have been shown to be strongly associated (Lapierre and Frenette 2009). This suggests the decrease in DOC

concentration downstream may alone be an indication of a shift to a greater proportion of autochthonous DOM.

## 6.2 Implications

Anthropogenic impacts such as agriculture and urbanization impact DOM characteristics in large rivers. These impacts were evident when comparing an impacted and a natural river; in this study the Grand River and the Burnt River were compared. In the Grand River human impacts accumulate changing DOM characteristics downstream compared to the natural Burnt River where DOM characteristics were more homogenous throughout the length. The seasonal cycle of DOM characteristics was greater in Grand River than Burnt River due to inorganic nutrients causing high primary production in warmer months. Generally, as the proportion of farmland increases the proportion of autochthonous DOM increases. Large STPs associated with urbanization release nutrients which increase primary production and stimulate macrophyte growth and increase autochthonous DOM. This labile DOM is more available to heterotrophic microorganisms which deplete oxygen, a threat to ecosystem health. In addition to impacts in the aquatic environment, human health is impacted by autochthonous DOM which has a higher disinfection byproduct forming potential as well containing more DON which forms more toxic nitrogen containing DBPs. DBPs are a concern to communities using an impacted water sources such as the Grand River as a drinking water source.

## 6.3 Future Work

To better understand the processes involved in the source and fate of DOM in the Grand River and Burnt River watersheds, this study could be expanded on in several ways. The temporal and spatial scales could be increased in length and resolution, respectively.

In terms of scale, the study could be expanded to include other watersheds with different water chemistries, landscape/subsurface geology and climate. Controlling for each of these in a study of many watersheds could improve the understanding of how DOM characteristics are affecting by each one. Within the watershed, tributaries could be better described with both point and non-point sources of autochthonous and allochthonous DOM identified. Small urban, suburban, agricultural and forested tributaries within the watershed could be described and compared. Small tributaries can be used to determine the DOM source to better understand how properties evolve downstream. Also, the time scale of the study could be increased to investigate

changes in DOM properties long term. These changes in scale could help to better understand the processes affecting DOM in a watershed and how they change over time.

Additionally, other techniques to describe DOM properties could be compared to those used in this study. Within the DOM pool there can be 1000s of different molecules; more techniques to investigate and describe these would be useful. $^{13}$C-NMR could be a useful technique to help understand the structures and functional groups of DOM molecules (Duan et al. 2007b). Additionally, there is great potential for use of liquid chromatography with organic carbon detection (LC-OCD) for study of DOM in aquatic ecosystems. LC-OCD can give the concentration of hydrophobic and hydrophilic DOC within a water sample. Also, concentrations can be determined for polysaccharides, proteins, humics, humic building blocks and amino acids. SUVA, a useful property for determining aromaticity, can be measured on all size fractions. This can help discriminate DOM sources. With LC-OCD, changes in DOM properties could be observed downstream and seasonally in a large river such as the Grand River. Using this technique, studies have shown that with little variability in DOC concentration there can be great variability in size fractions throughout the seasons observed (Fischer et al. 2002, Frimmel 1998, Schwendenmann and Veldkamp 2005). This has many advantages over the high-performance size exclusion chromatography (HPSEC) used to determine average apparent molecular weight in chapter 3 and 4. Using HPSEC only an average apparent molecular weight can be determined compared to LC-OCD which can quantify size fractions quickly with a small volume of sample. The largest advantage of LC-OCD over HPSEC is the elemental analysis of size fractions compared to UV absorbance which will not measure all types of molecules. Both $^{13}$C-NMR and LC-OCD could be compared to other techniques used in this study such as stable isotopic analysis and fluorescence.
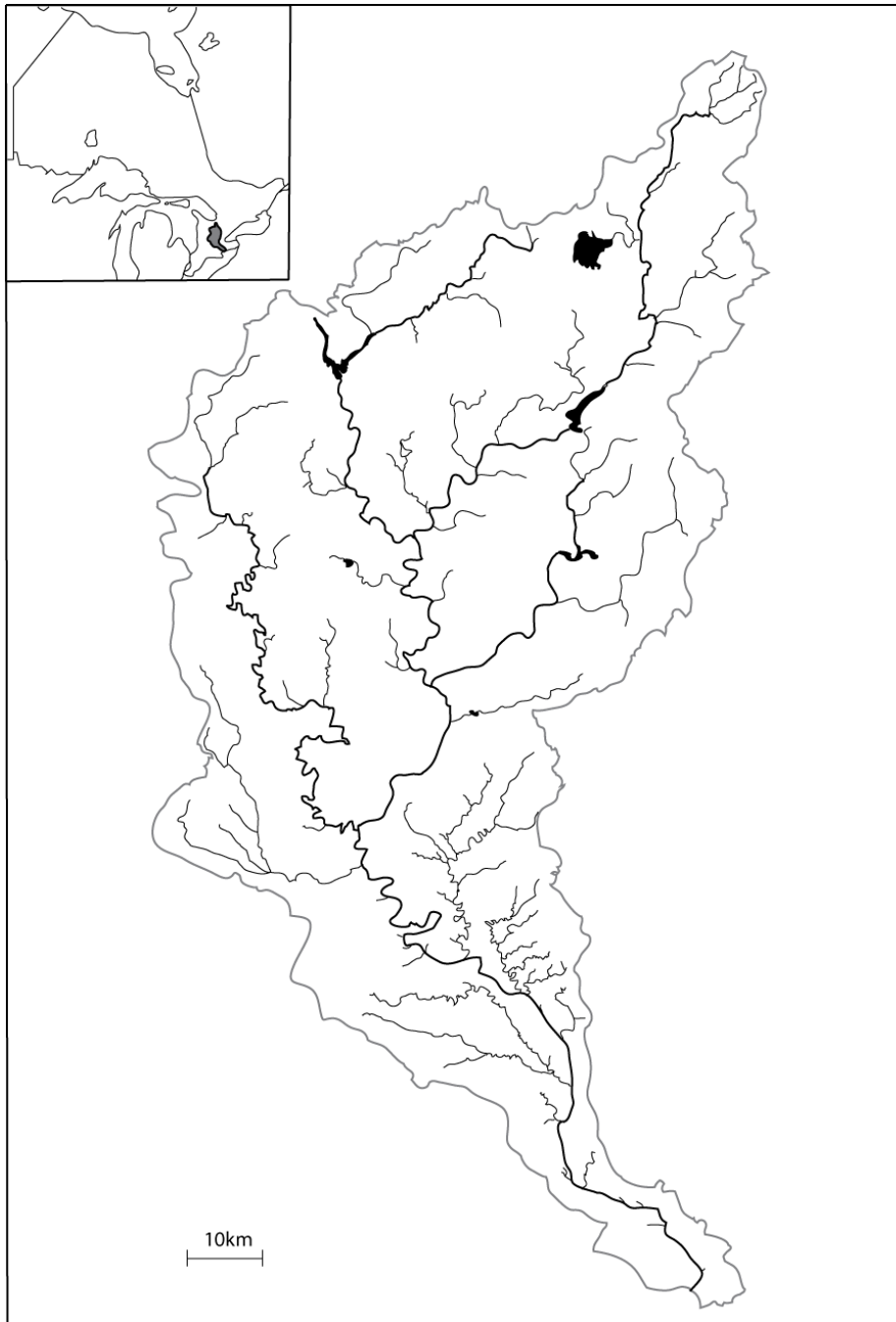
# Tables and Figures



Figure 1.1: Grand River watershed

Table 2.1: Total annual precipitation and mean annual temperature at Waterloo Wellington Weather station (Environment Canada, National Climate and Information Archive).

| Year | Total precipitation (mm) | Mean Annual Temperature (°C) |
|---|---|---|
| 1981 | 823.8 | 6.48 |
| 1982 | 1063.8 | 6.20 |
| 1983 | 969.6 | 7.18 |
| 1984 | 946.4 | 6.54 |
| 1985 | 1186.4 | 6.53 |
| 1986 | 1064.6 | 6.73 |
| 1987 | 943.8 | 7.62 |

Table 2.2: Comparison of seasonal discharge, DOC, DON, DOC/DON in the headwaters (upper Conestogo River) and lower river (Grand River at Glen Morris) during 1981 to 1988

| | | Discharge $(m^3s^{-1})$ | Mean Concentration $(mg\ l^{-1})$ | | | Average Daily Load $(10^3\ kg)$ | |
|---|---|---|---|---|---|---|---|
| | | | DOC | DON | DOC/ DON | DOC | DON |
| Headwaters | spring | 11.0 | 6.5 | 0.80 | 10.6 | 5.1 | 1.5 |
| (Upper Conestogo | summer | 3.1 | 8.0 | 0.81 | 12.1 | 2.1 | 0.30 |
| River) | fall | 2.1 | 6.8 | 0.66 | 12.4 | 1.3 | 0.13 |
| | winter | 8.6 | 5.6 | 0.65 | 11.3 | 3.4 | 0.59 |
| Lower-Central River | spring | 68.1 | 5.0 | 0.74 | 8.3 | 28 | 5.0 |
| (Grand River | summer | 21.9 | 5.8 | 0.78 | 8.8 | 11 | 1.4 |
| at Glen Morris) | fall | 50.0 | 6.1 | 0.83 | 9.0 | 27 | 4.0 |
| | winter | 37.1 | 5.0 | 0.65 | 10.7 | 16 | 2.6 |

Table 2.3: Mean annual fluxes of organic carbon and nitrogen for 7 sites on the Grand River and its tributaries using PWQMN data from 1981 to 1987 and % farmland from (GRCA 2005). Qi is mean annual discharge based on flows on the days of sampling and Qa is the mean annual discharge based on daily flows throughout the entire year. (H-headwaters, UC-upper central, LC-lower central, L-lower)

| | | Drainage Area (km$^2$) | % Farmland | Altitude ASL (m) | Mean Discharge (m$^3$s$^{-1}$) | | Qi/Area 10$^{-2}$ (m$^3$s$^{-1}$km$^{-2}$) | Mean Concentration (mg l$^{-1}$) | | DOC/ DON | Mean Annual Flux (10$^3$ kg) | | Flux per area (kg km$^{-2}$ yr$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Qi | Qa | | DOC | DON | | DOC | DON | DOC | DON |
| H | Upper Conestogo | 272 | 62 | 407 | 5.78 | 3.69 | 1.37 | 6.80 | 0.74 | 11.6 | 877 | 190 | 3220 | 697 |
| | Canagagigue Creek | 98 | 85* | 355 | 1.96 | 1.61 | 2.00 | 4.85 | 1.15 | 6.0 | 306 | 67.9 | 3120 | 693 |
| UC | Grand River below Belwood | 800 | 51 | 406 | 10.96 | 10.18 | 1.13 | 7.76 | 0.62 | 12.7 | 2690 | 223 | 3360 | 279 |
| | Speed River | 600 | 86 | 296 | 6.77 | 6.61 | 2.12 | 5.58 | 0.68 | 9.9 | 1200 | 141 | 2000 | 235 |
| LC | Nith River | 1080 | 63 | 220 | 15.06 | 14.09 | 1.26 | 4.22 | 0.67 | 8.3 | 2300 | 457 | 2120 | 423 |
| | Grand River at Glen Morris | 3600 | 83 | 265 | 45.36 | 44.38 | 1.39 | 5.45 | 0.75 | 9.1 | 7590 | 1198 | 2110 | 333 |
| L | Grand River at York | 5910 | 61 | 181 | 71.95 | 70.34 | 1.22 | 5.14 | 0.74 | 8.6 | 11800 | 1940 | 1990 | 329 |

*Estimated from 1999 GRCA land survey

Table 2.4: Results of two-sample t-test assuming unequal variance for the comparison of seasonal DOC, DON and DOC/DON in the headwaters (upper Conestogo River) and lower-central river (Grand River at Glen Morris) during 1981 to 1988; P-value <0.05 significant difference

| | Headwaters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | DOC | | | DON | | | DOC/DON | | |
| | Spring | Summer | Fall | Spring | Summer | Fall | Spring | Summer | Fall |
| Spring | - | | | - | | | - | | |
| Summer | 0.000 | - | | N.S. | - | | N.S. | - | |
| Fall | 0.000 | N.S. | - | N.S. | N.S. | - | N.S. | N.S. | - |
| Winter | N.S. | 0.000 | 0.000 | N.S. | N.S. | 0.032 | 0.039 | N.S. | N.S. |

| | Lower-Central River | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | DOC | | | DON | | | DOC/DON | | |
| | Spring | Summer | Fall | Spring | Summer | Fall | Spring | Summer | Fall |
| Spring | - | | | - | | | - | | |
| Summer | 0.000 | - | | N.S. | - | | N.S. | - | |
| Fall | 0.000 | N.S. | - | N.S. | N.S. | - | N.S. | N.S. | - |
| Winter | N.S. | 0.000 | 0.000 | N.S. | N.S. | 0.032 | 0.039 | N.S. | N.S. |

Figure 2.1: Grand River watershed boundary and sites
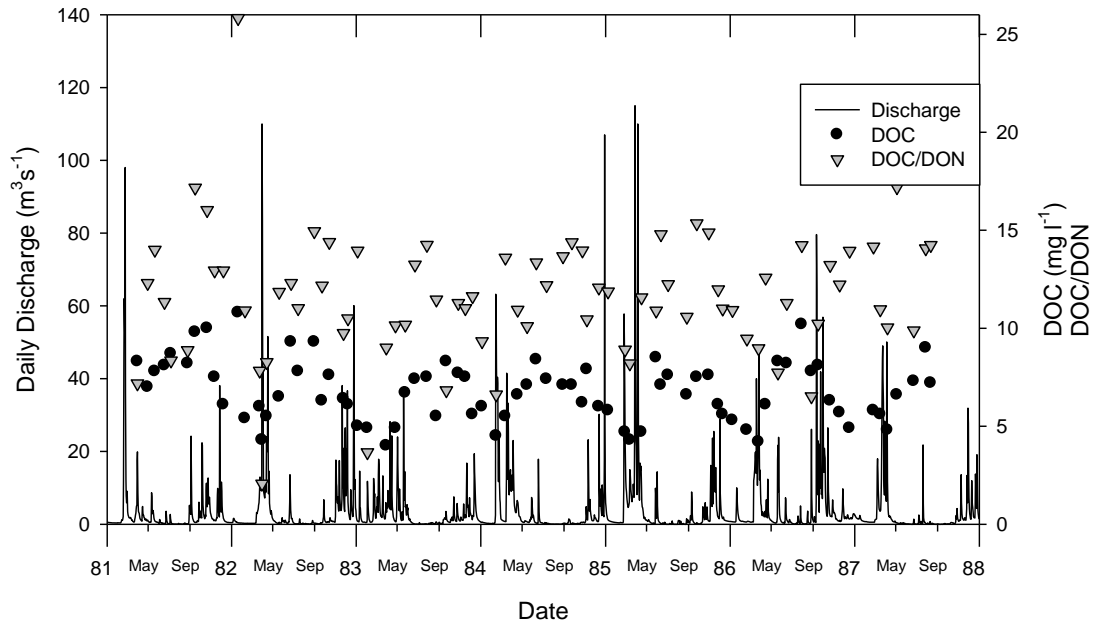
Figure 2.2: Continuous discharge and monthly DOC and DOC/DON from 1981 to 1988 in the upper Conestogo River
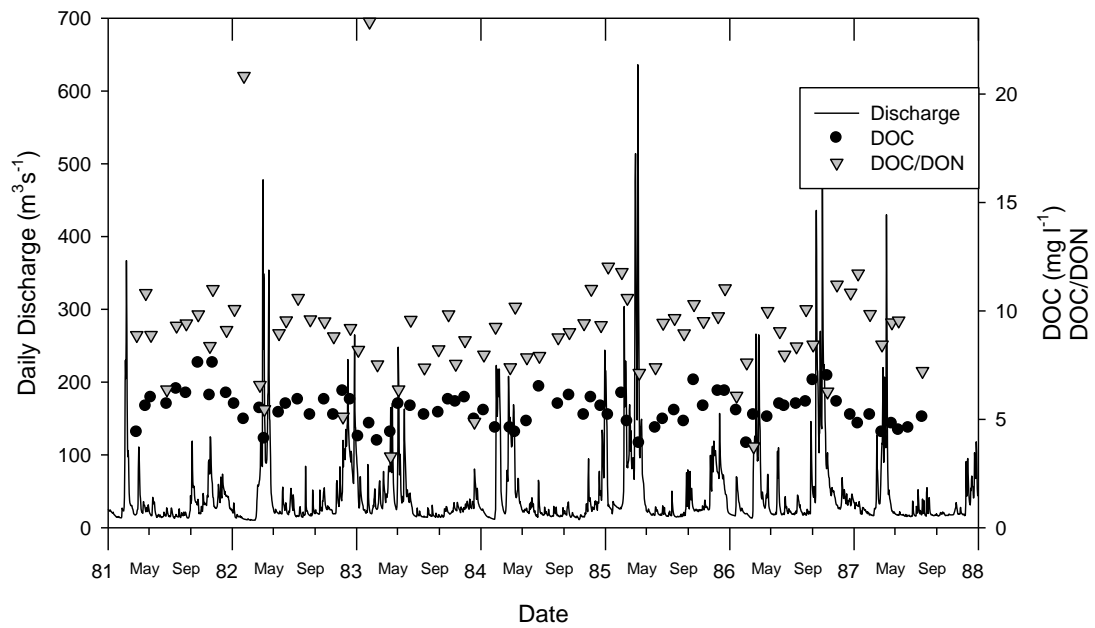


Figure 2.3: Continuous discharge and monthly DOC and DOC/DON from 1981 to 1988 in the lower-central Grand River at Glen Morris
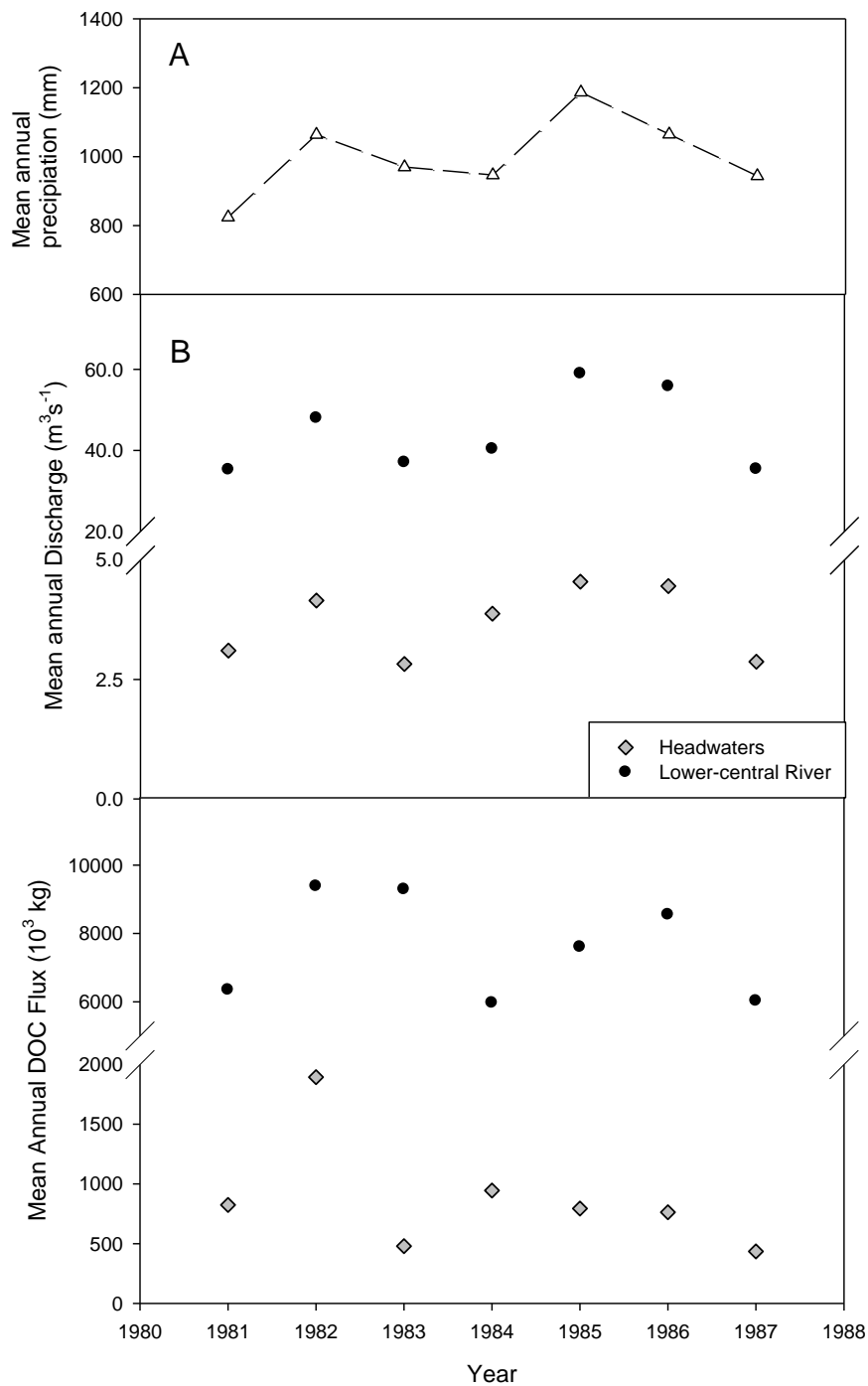
Figure 2.4: Mean annual precipitation (A), mean annual discharge (B) and DOC flux (C) in the headwaters measured in the upper Conestogo River and lower-central Grand River measured near Glen Morris.
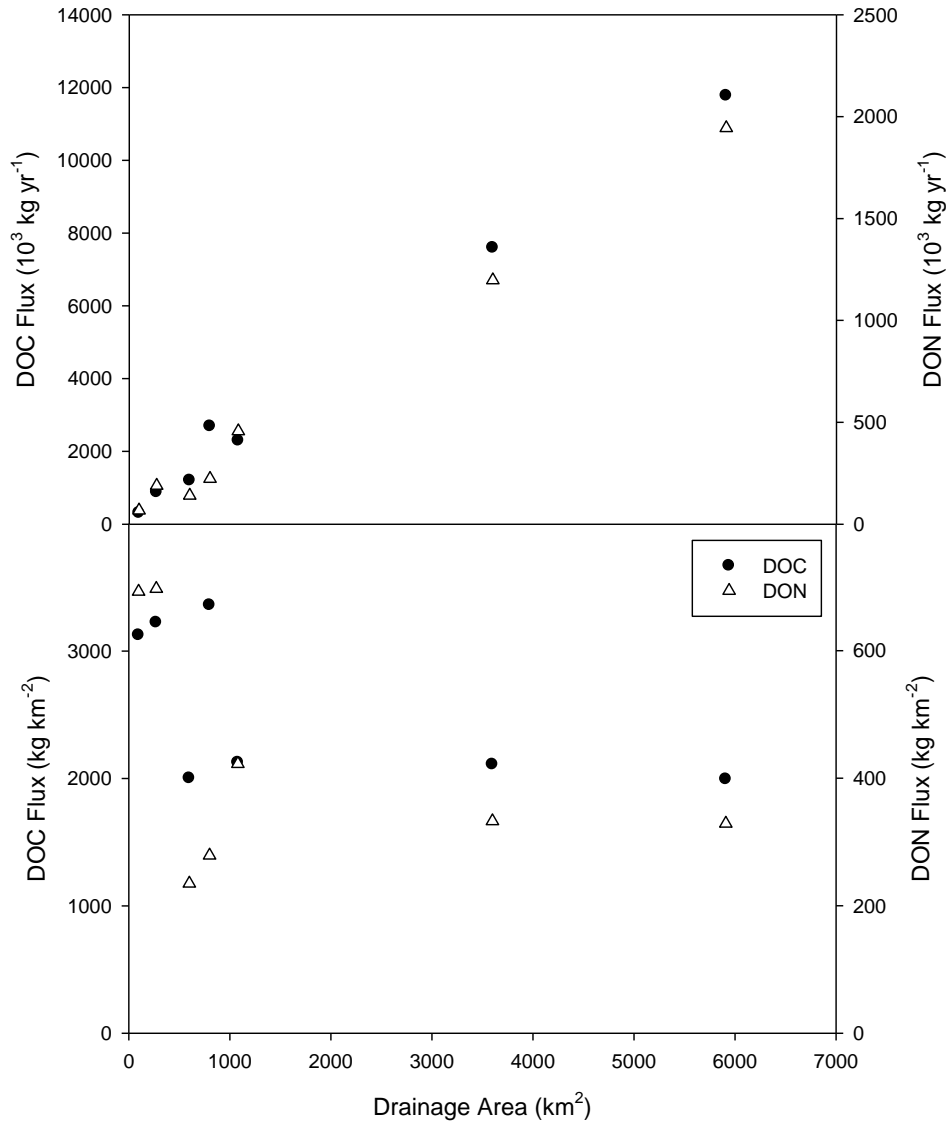
Figure 2.5: DOC and DON flux and flux per area for 7 sites on the Grand River and its tributaries from 1981 to 1987
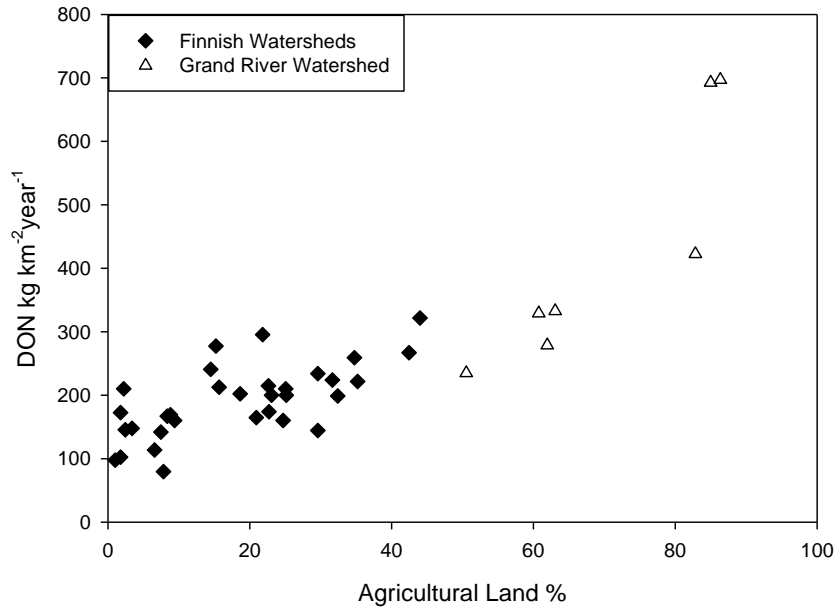
Figure 2.6: Percent agricultural land and mean annual DON export per area in Finnish watersheds with less than 5% lake area from 1995 to 1999 (from Mattsson et al. 2005) and 7 subcatchments of the Grand River watershed from 1981 to 1987
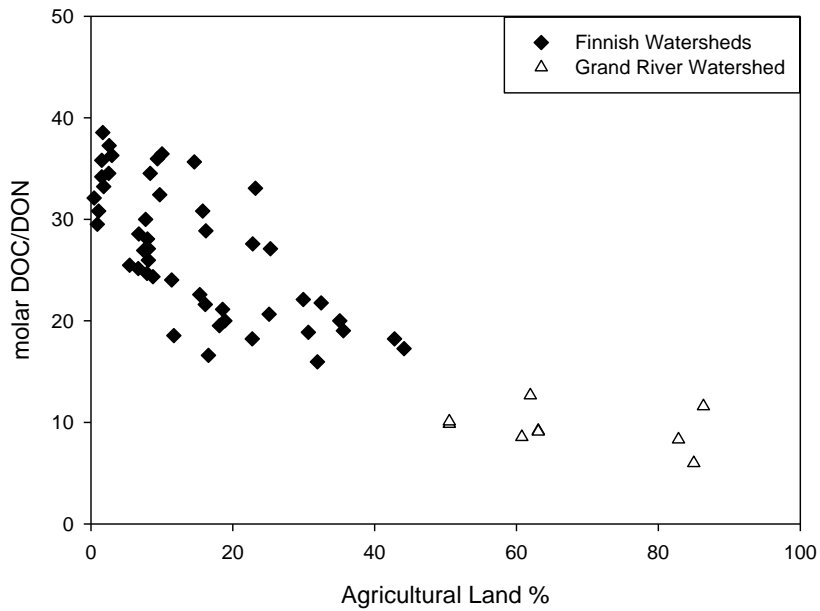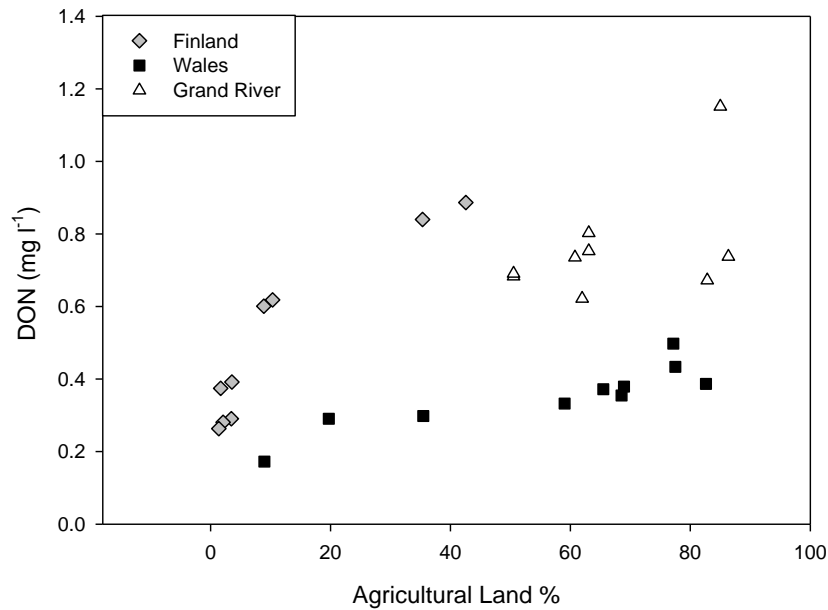


Figure 2.7: Percent agricultural land and mean molar DOC/DON in 86 Finnish watersheds from 1995 to 1999 (from Mattsson et al. 2005) and 9 subcatchments of the Grand River watershed from 1981 to 1987

Figure 2.8: Percent agricultural land and DON in 9 Finnish and 10 Welsh watersheds in 2001 and 2002 (from Mattsson et al. 2009) and 9 subcatchments of the Grand River watershed from 1981 to 1987
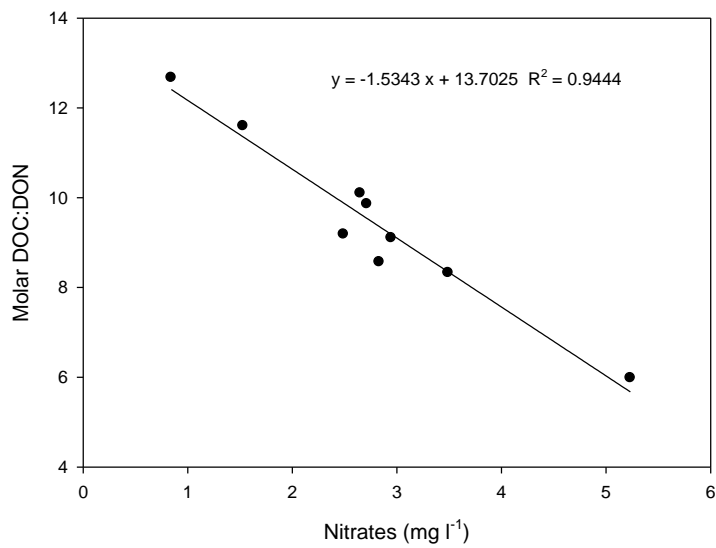


Figure 2.9: Relationship between mean molar DOC/DON and mean nitrates ($NO_2^-$ + $NO_3^-$) in 9 subcatchments in the Grand River watershed from 1981 to 1987
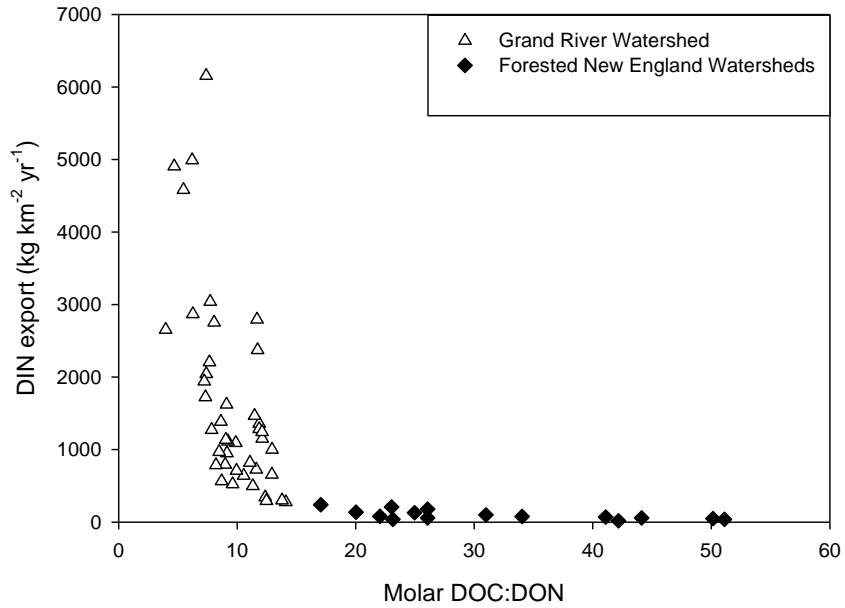
Figure 2.10: Relationship between molar DOC/DON and annual export of DIN ($NH_4^+ + NO_2^- + NO_3^-$) in 9 forested watersheds in New England (from Cambell et al. 2000) and 7 subcatchments in the Grand River watershed

Table 3.1: DOM Indicator Summary

| | Range | Downstream Trend | Literature Values | Allochthonous Vs. Autochthonous | Seasonal |
|---|---|---|---|---|---|
| DOC/DON | 4.4 – 36 | Decreasing until 143km Increasing after 143km | Allo ~88[a] Auto ~20[a] | Autochthonous | No difference |
| FI | 1.1 – 1.8 | Increasing | Allo ~1.4[b] Auto ~1.9[b] | Both | No difference |
| SUVA | 1.2 – 3.7 | Decreasing/No Trend | Allo ~7.4[c] Auto ~1[c] | Autochthonous | Highest in February |
| MW | 1060 – 2030 | Decreasing | Allo <1500[d] Auto ~1000[d] | Autochthonous (except February) | Highest in February |
| a250/a365 | 3 –13 | No Trend | ND | NA | Lowest in February |
| Protein-like/DOC | 0.17 – 3.9 | Increasing | ND | NA | Lowest in February |
| Humic-like/DOC | 2.2 – 10.7 | No Trend | ND | NA | September > February > June > October |
| P/HFlu | 0.06 – 0.52 | Increasing | ND | NA | September/October > June/February |
| DON- δ15N | -0.8 – 9.5 | Increasing | Allo ~0[e] Auto <2[e] | Autochthonous (except some headwater) | Lowest in February |

[a](Mcknight et al. 1991)
[b](McKnight et al. 2001)
[c](Her et al. 2004)
[d](Nguyen et al. 2010)
[e](HOOD ET AL. 2005)

Table 3.2: Pearson Correlation Summary

| | Distance | DOC | DOC/ DON | TN | NO$_3$ | NH$_4$ | DON | FI | SUVA | MW | a250/ a365 | NORM PROT | NORM HUM | PROT HUM | C13 | N15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Distance | 1 | | | | | | | | | | | | | | | |
| DOC | -0.777 | 1 | | | | | | | | | | | | | | |
| DOC/DON | N.S. | 0.521 | 1 | | | | | | | | | | | | | |
| TN | 0.486 | N.S. | N.S. | 1 | | | | | | | | | | | | |
| NO3 | 0.535 | N.S. | N.S. | 0.981 | 1 | | | | | | | | | | | |
| NH4 | N.S. | N.S. | N.S. | N.S. | N.S. | 1 | | | | | | | | | | |
| DON | N.S. | N.S. | -0.611 | 0.594 | 0.572 | N.S. | 1 | | | | | | | | | |
| FI | 0.469 | -0.48 | N.S. | N.S. | N.S. | N.S. | N.S. | 1 | | | | | | | | |
| SUVA | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | 1 | | | | | | | |
| MW | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | 0.705 | 1 | | | | | | |
| a250/a365 | N.S. | N.S. | N.S. | -0.558 | -0.567 | N.S. | N.S. | N.S. | -0.67 | -0.669 | 1 | | | | | |
| NORM_PROT | 0.771 | -0.874 | -0.398 | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | -0.533 | N.S. | 1 | | | | |
| NORM_HUM | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | 0.486 | N.S. | N.S. | N.S. | 1 | | | |
| PROT_HUM | 0.457 | -0.564 | -0.305 | N.S. | N.S. | N.S. | N.S. | N.S. | -0.793 | -0.737 | 0.573 | 0.738 | N.S. | 1 | | |
| C13 | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | 1 | |
| N15 | 0.679 | -0.669 | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | 0.709 | N.S. | N.S. | N.S. | 1 |
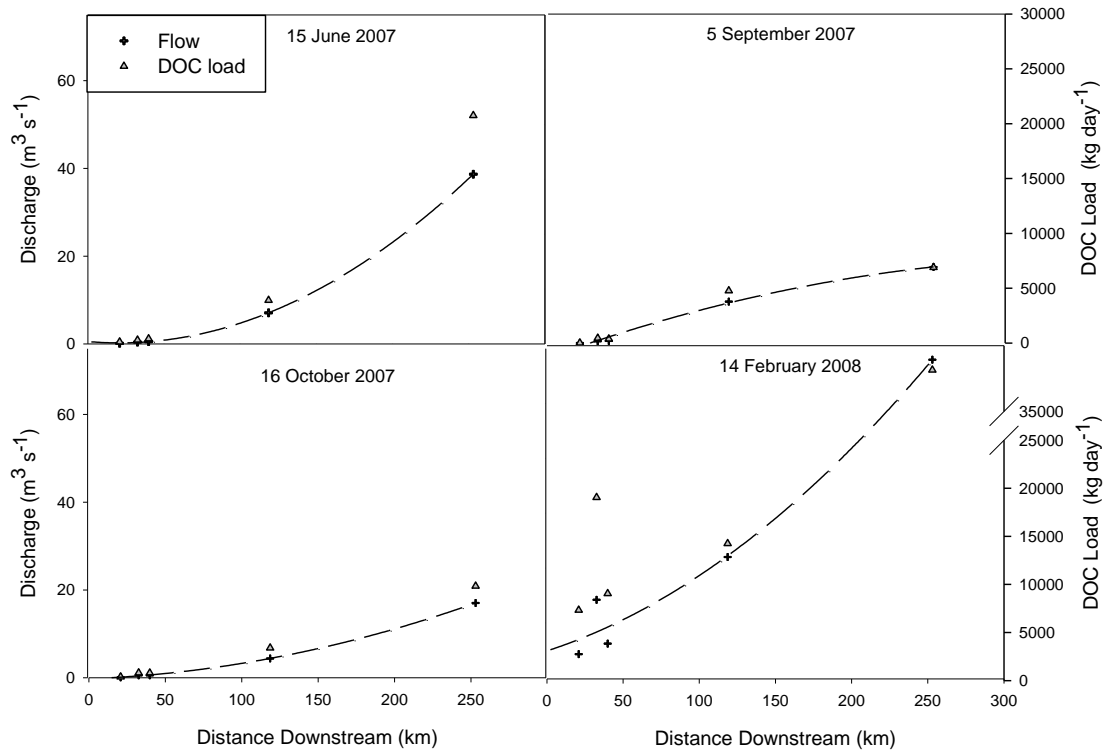
Figure 3.1: Water discharge and DOC load for 5 sites along the Grand River during surveys on June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008. The dashed line represents a fitted quadratic regression of discharge ($r^2 = 0.97$, 1.00, 0.95, 1.00). Discharge data provided by the GRCA.
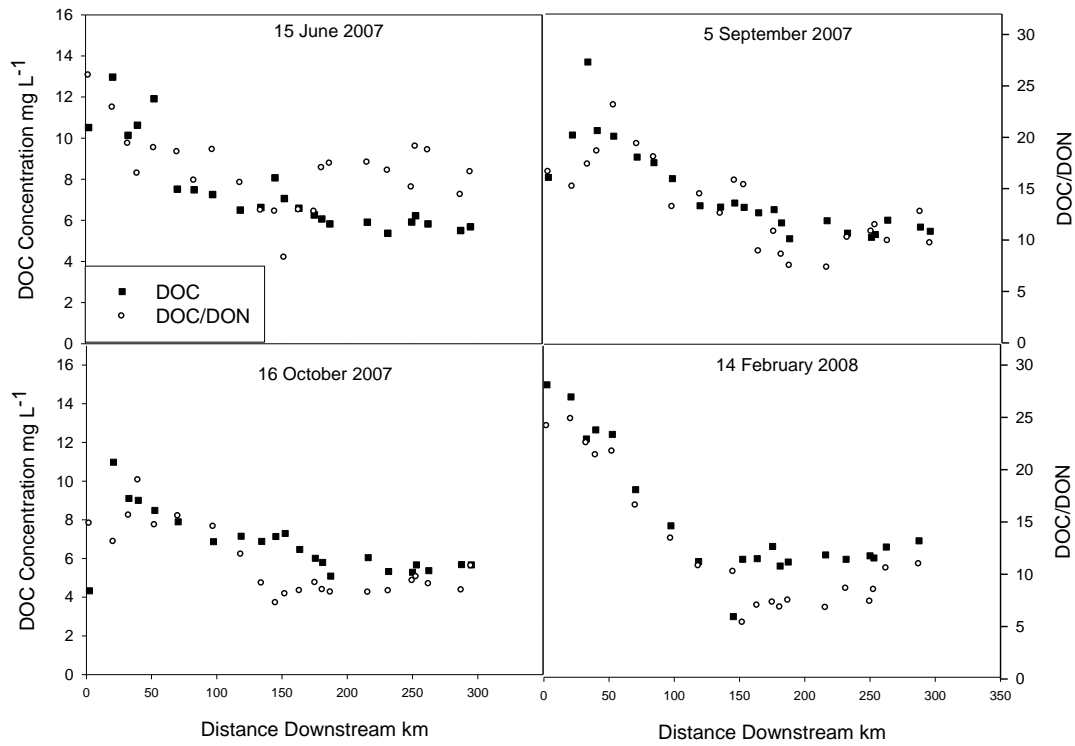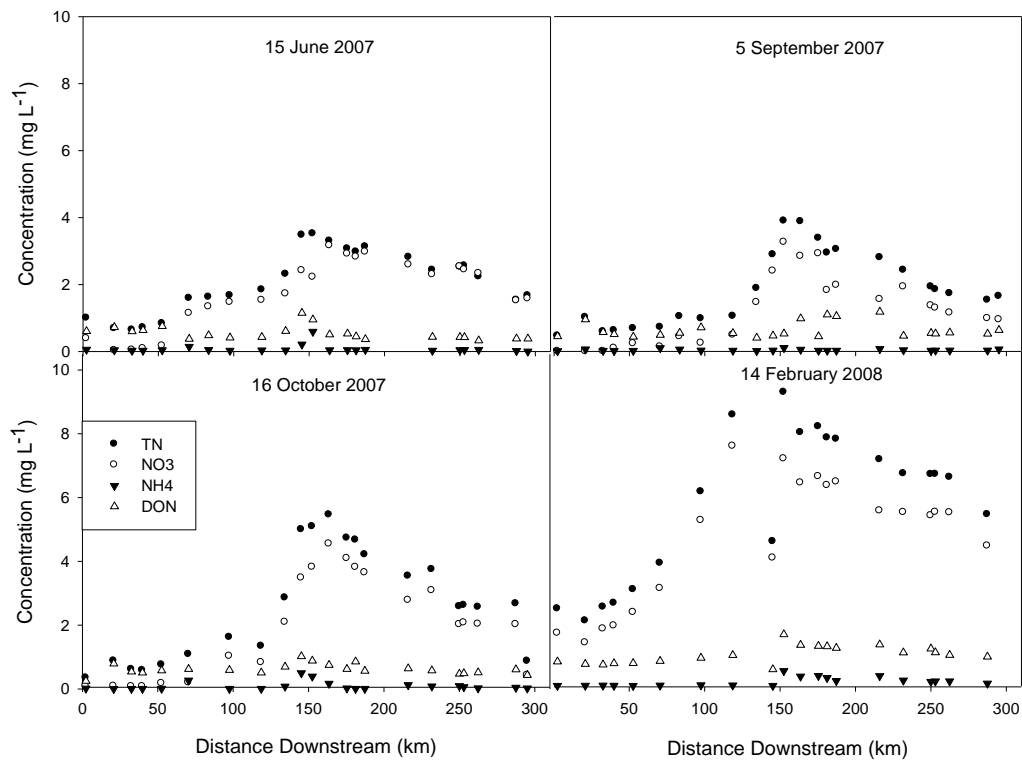
Figure 3.2: DOC concentrations and ratio of DOC to DON concentrations for 23 sites along the Grand River during surveys on June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.

Figure 3.3: Total disolved nitrogen (TDN), nitrate ($NO_3^-$), ammonia/ammonium ($NH_4^+$) and dissolved organic nitrogen (DON) for 23 sites along the Grand River during surveys on June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.
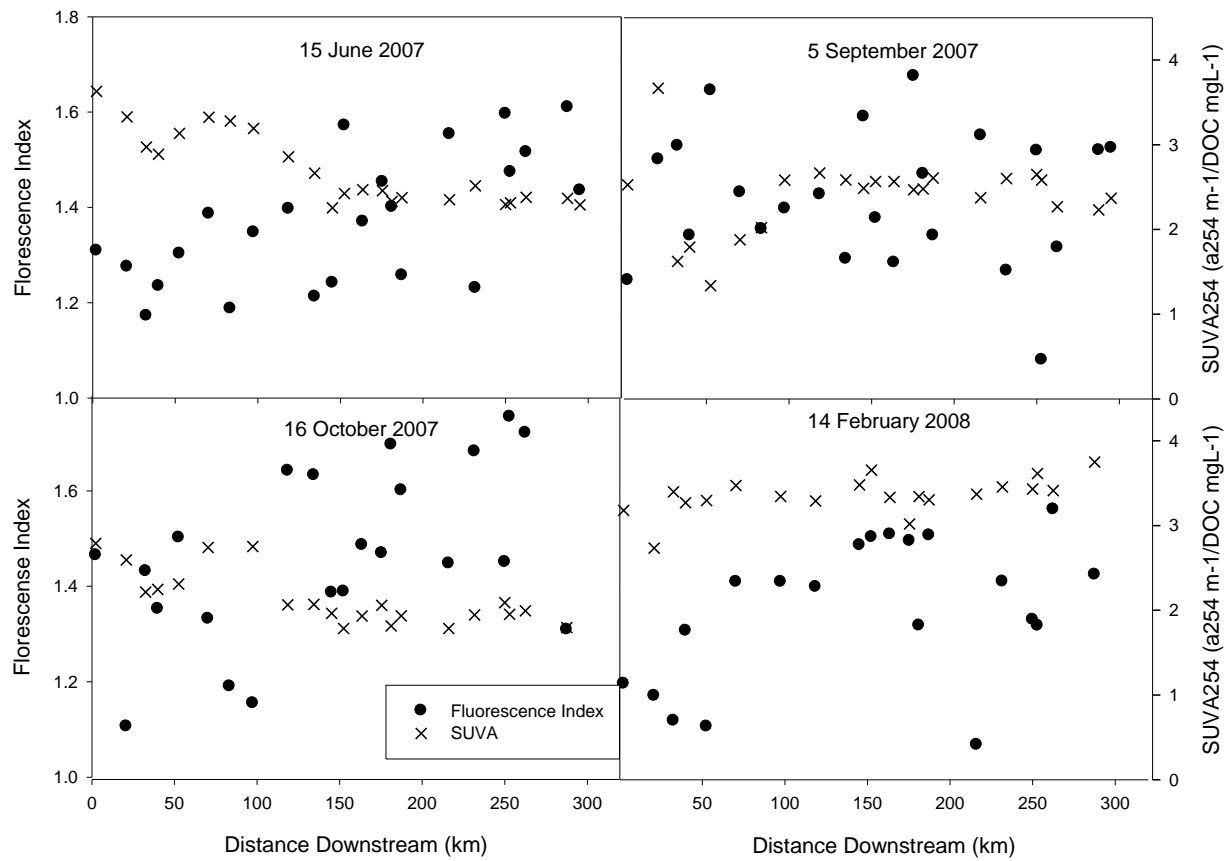
Figure 3.4: Fluorescence index and SUVA for 23 sites along the Grand River during surveys on June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.
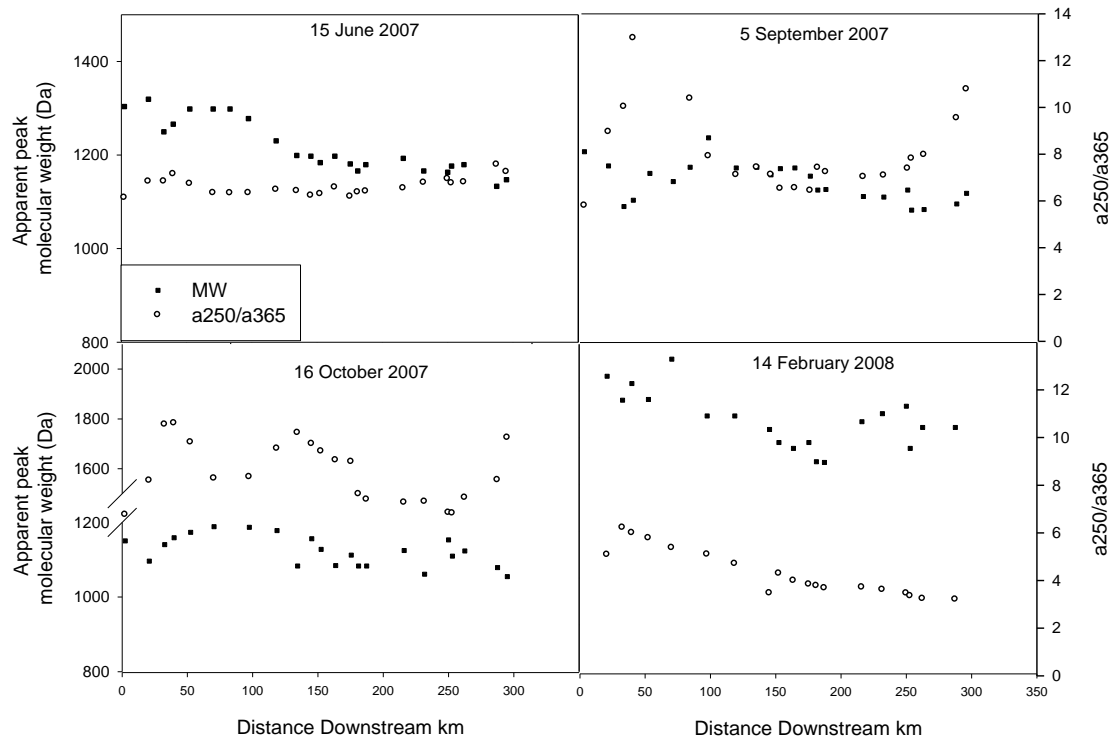
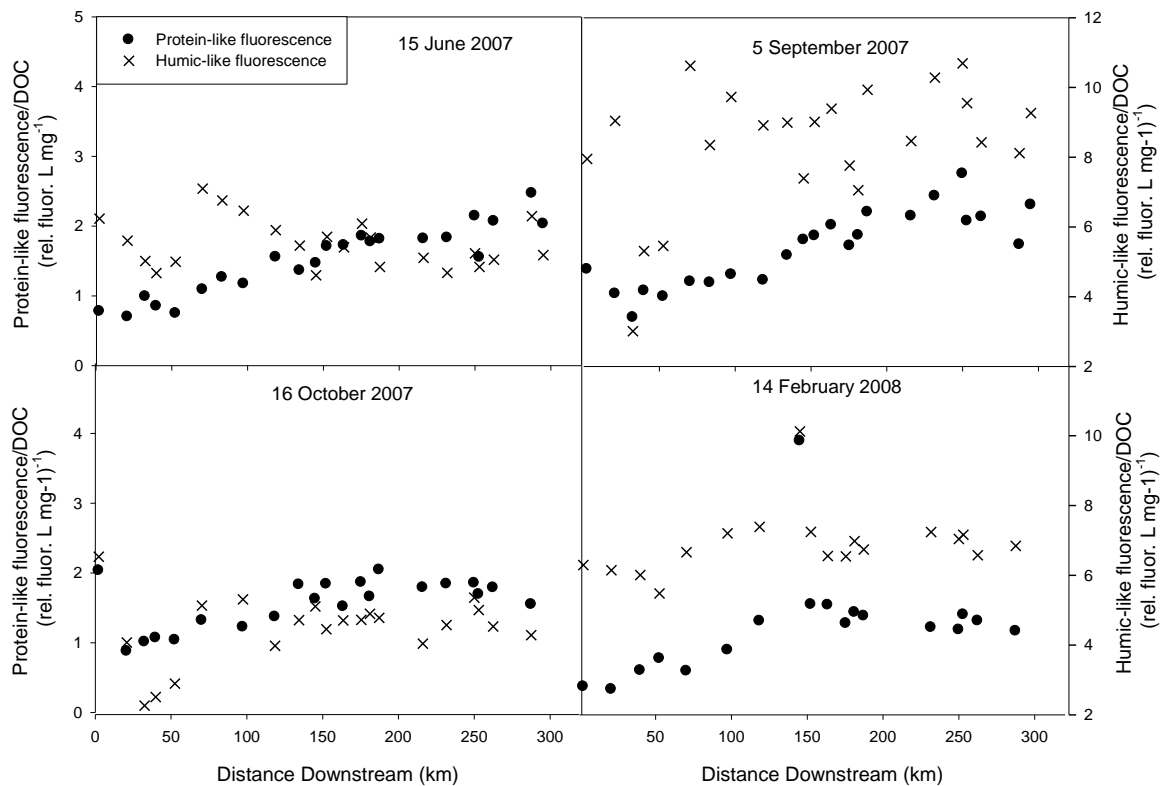Figure 3.5: Peak apparent molecular weight and absorbance 250 nm/365 nm ratio (an estimate of the proportion of low-molecular-weight to high-molecular-weight DOM) for 23 sites along the Grand River during surveys on June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.

Figure 3.6: Protein-like florescence and humic-like fluorescence normalized to DOC concentration for 23 sites along the Grand River during surveys on June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.
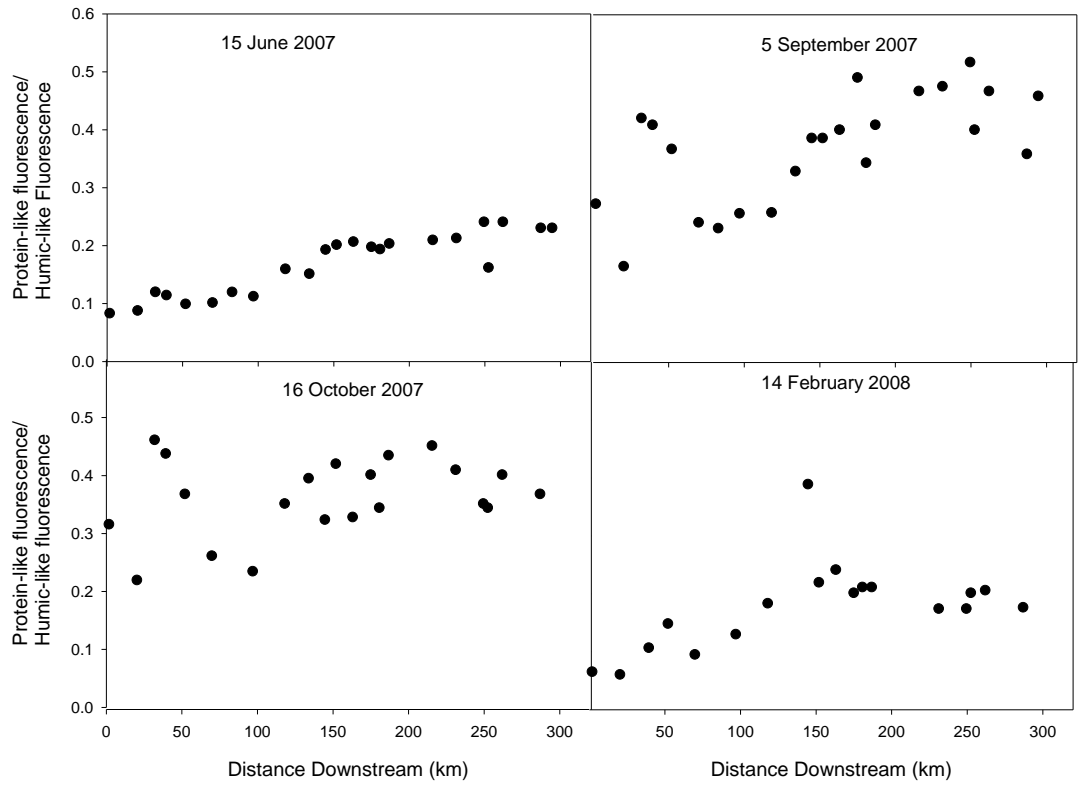
Figure 3.7: The ratio of protein-like florescence to humic-like fluorescence for 23 sites along the Grand River during surveys on June 15 June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.
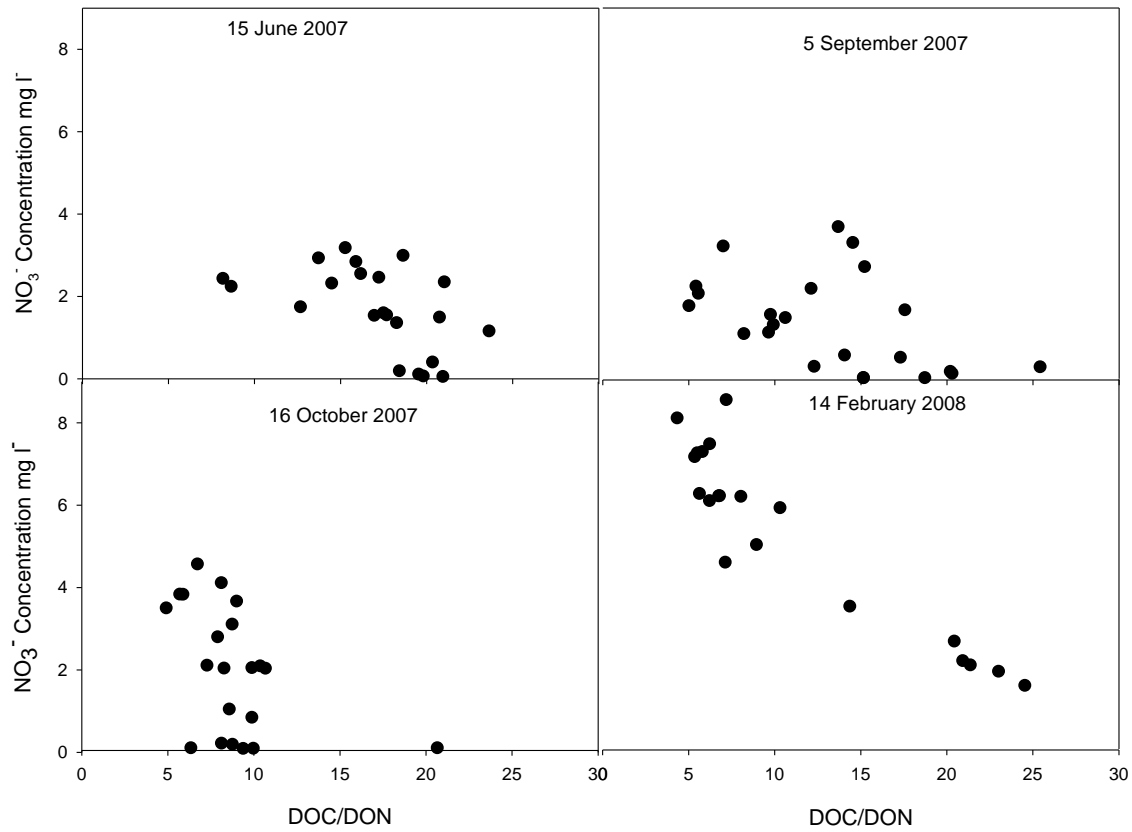
Figure 3.8: Relationship between DOC/DON and nitrate (NO$_3$$^-$) in the Grand River during surveys on June 15 June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.
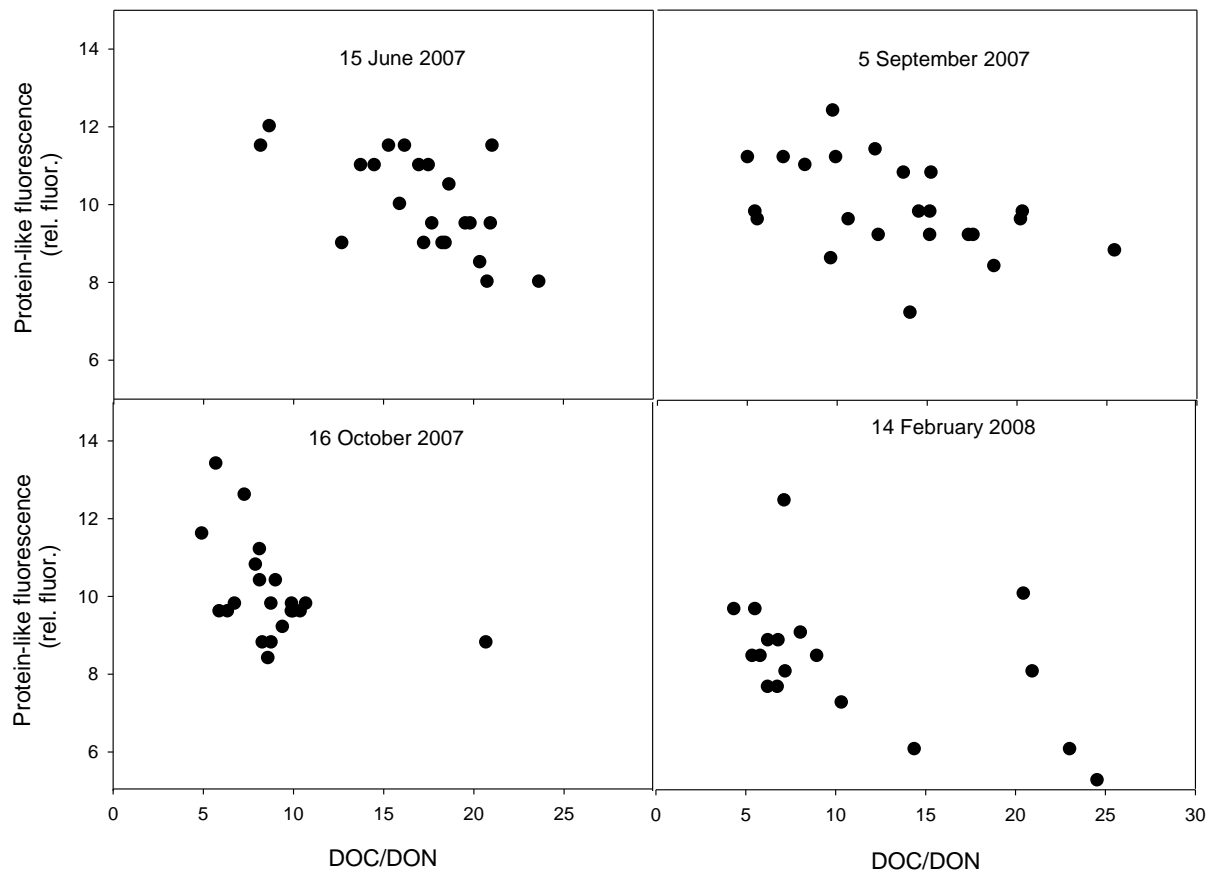
Figure 3.9: Relationship between DOC/DON and protein-like florescence in the Grand River during surveys on June 15 June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.
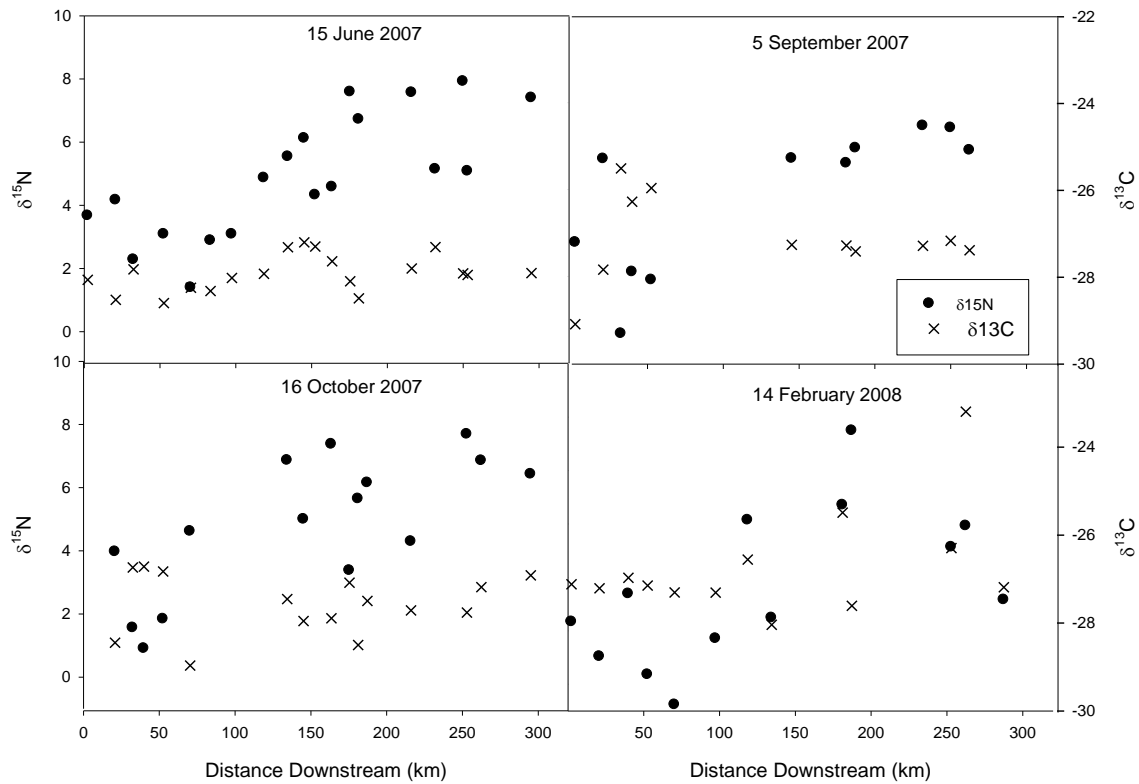
Figure 3.10: $\delta^{13}C$ and $\delta^{15}N$ of DOM for 23 sites along the Grand River during surveys on June 15 June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.



Figure 3.11: Relationship between protein-like fluorescence/DOC and DON-$\delta N^{15}$ in the Grand River during 2007-2008.

Figure 3.12: Relationship between protein-like fluorescence/DOC and DON-δN15 in the Grand River during surveys on June 15 June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.



Figure 3.13: δ15N of NO3- and δ15N of DON for 23 sites along the Grand River during surveys on June 15 June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.
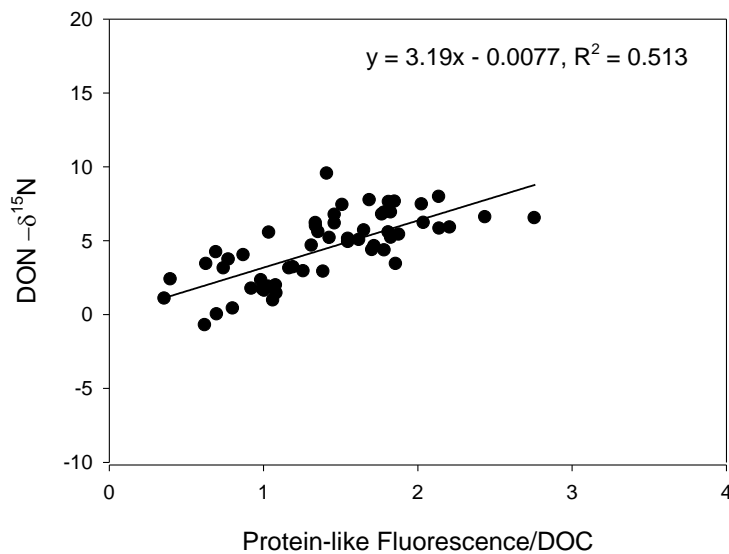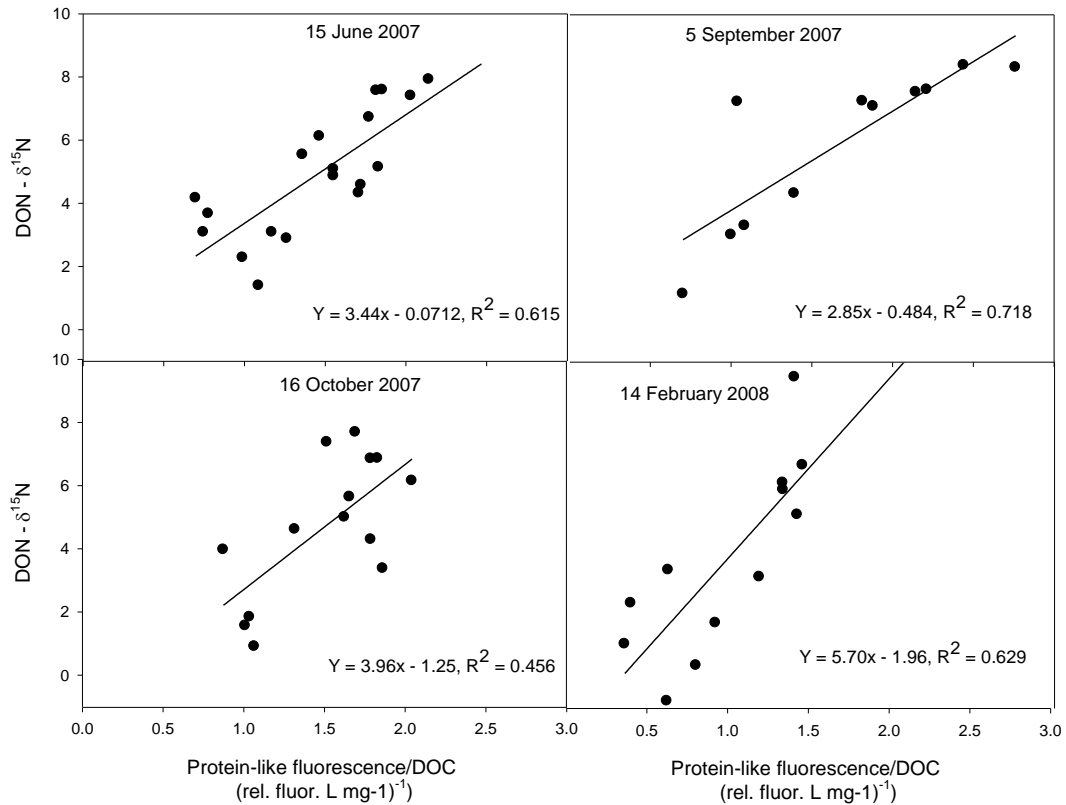
Figure 3.14: DOC/DON and δ15N of DON for 23 sites along the Grand River during surveys on June 15 June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.
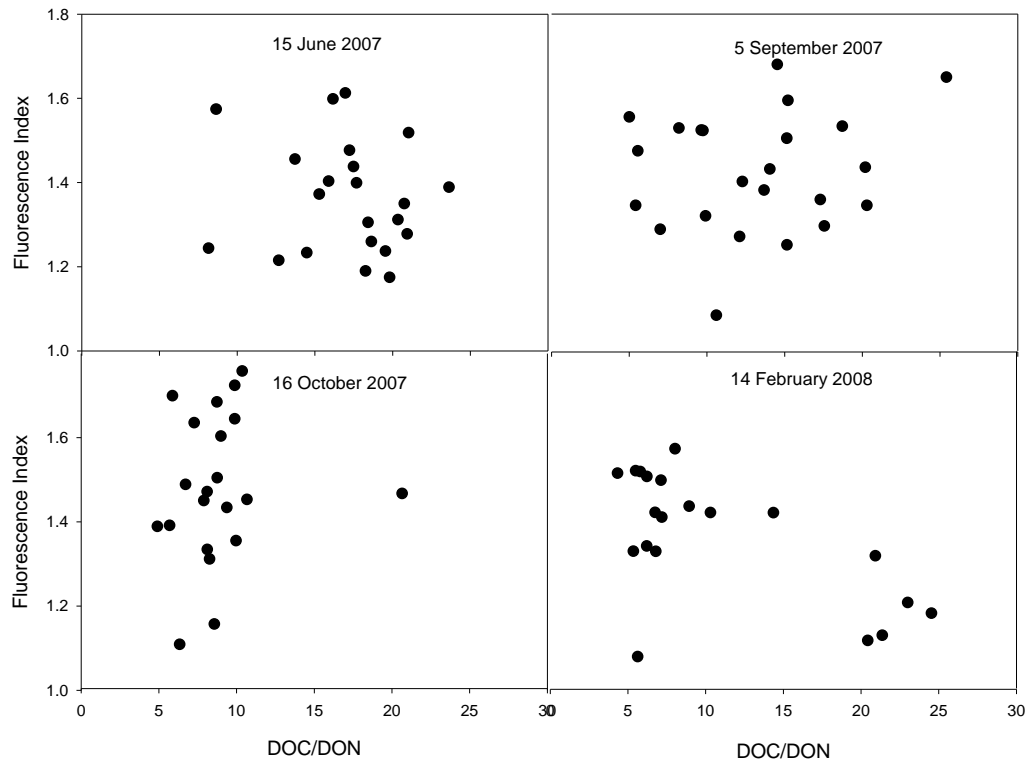
Figure 3.15: DOC/DON and FI for 23 sites along the Grand River during surveys on June 15 June 15th 2007, September 5th 2007, October 16th 2007, and February 14th 2008.
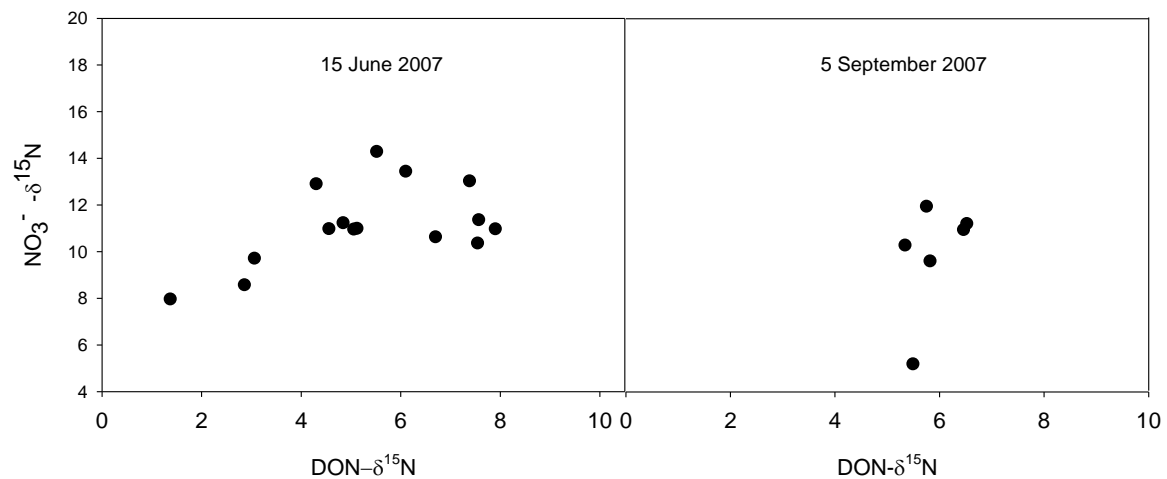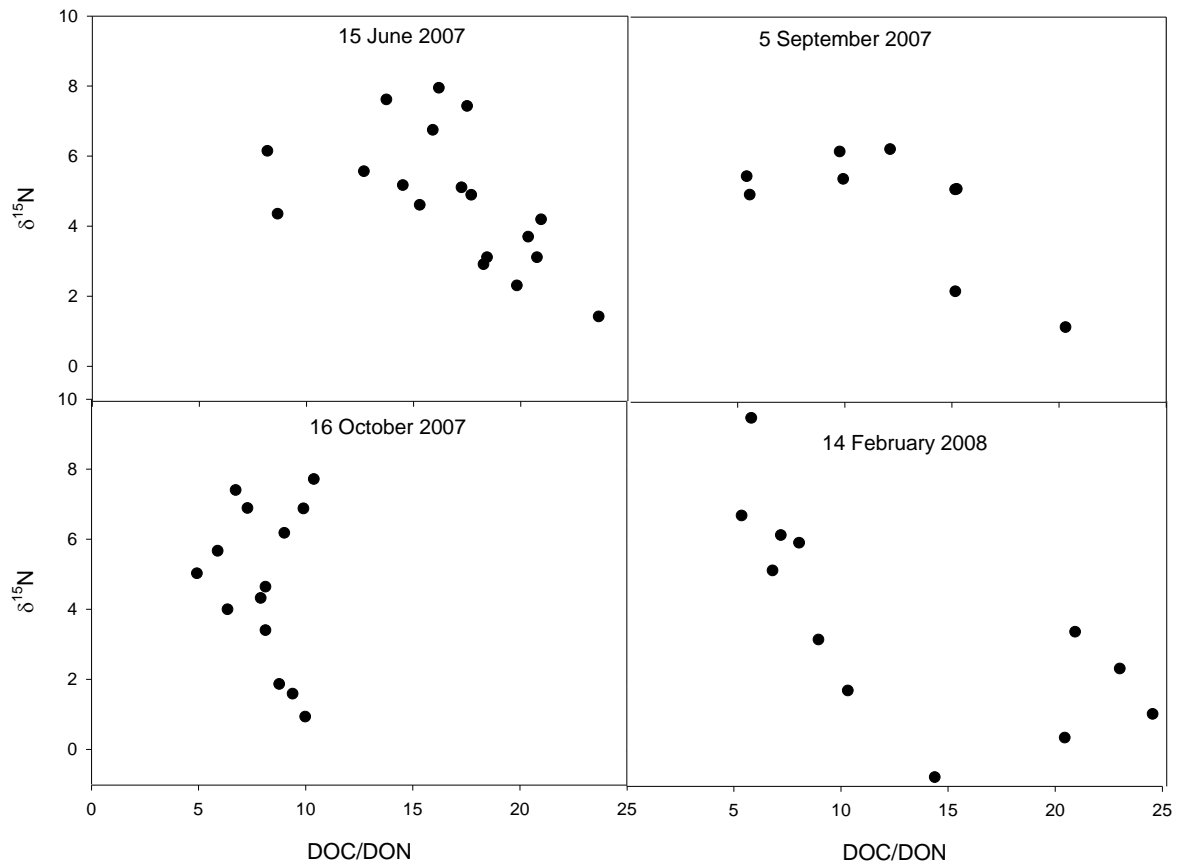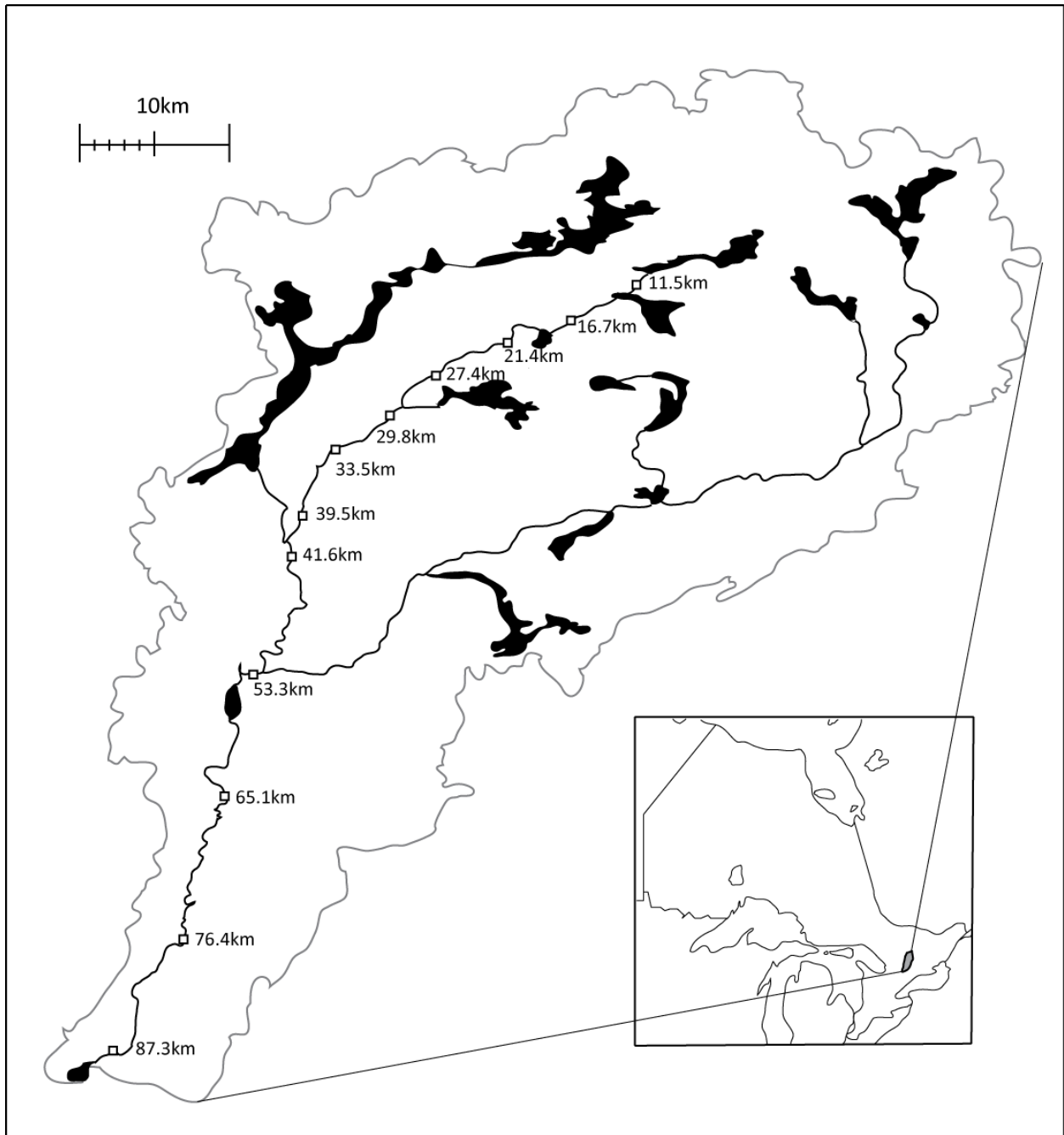
Figure 4.1: Burnt River Watershed. Squares represent sampling locations. Labels indicate river kilometre of sites.
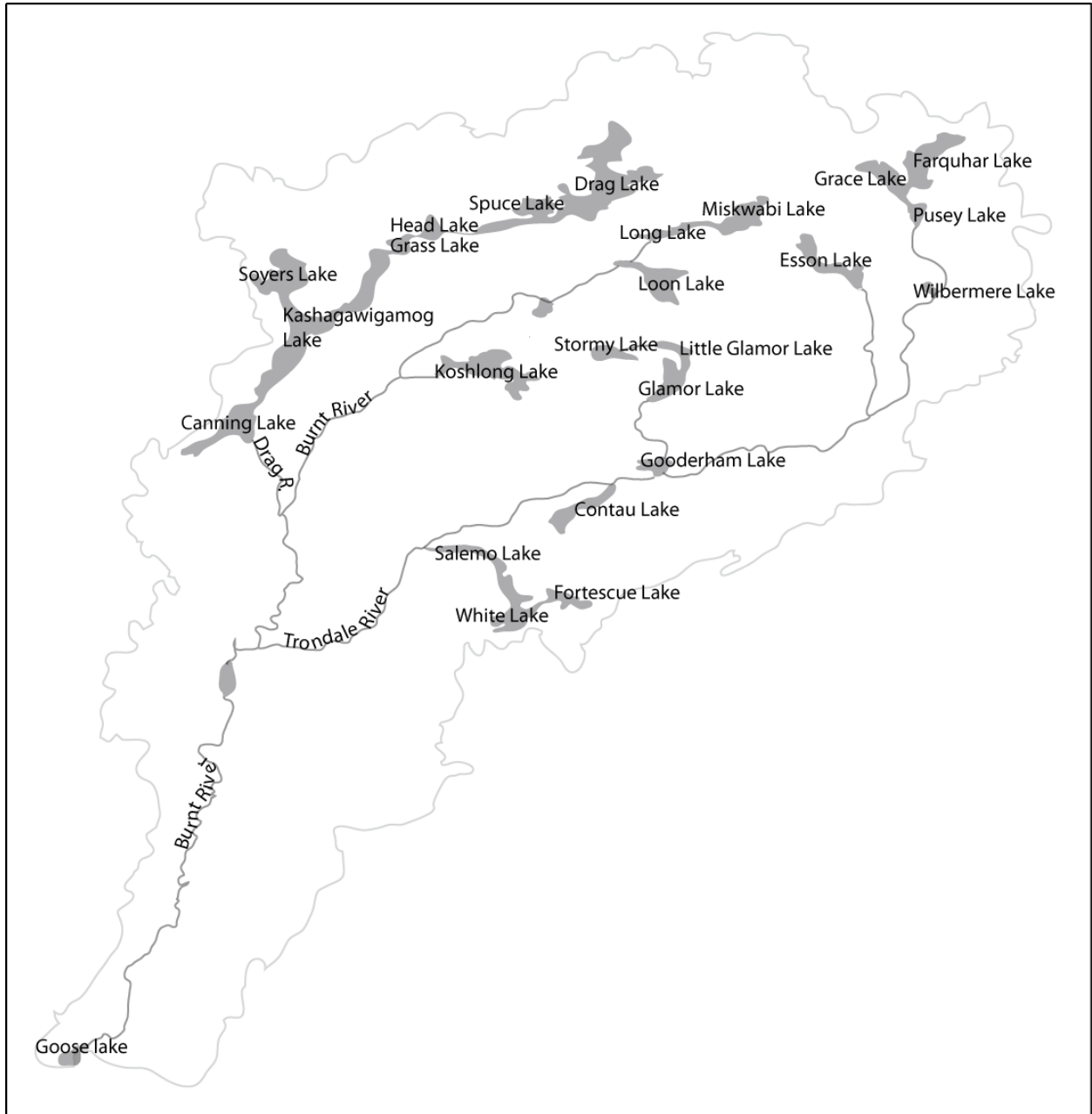
Figure 4.2: Major lakes and tributaries in the Burnt River watershed
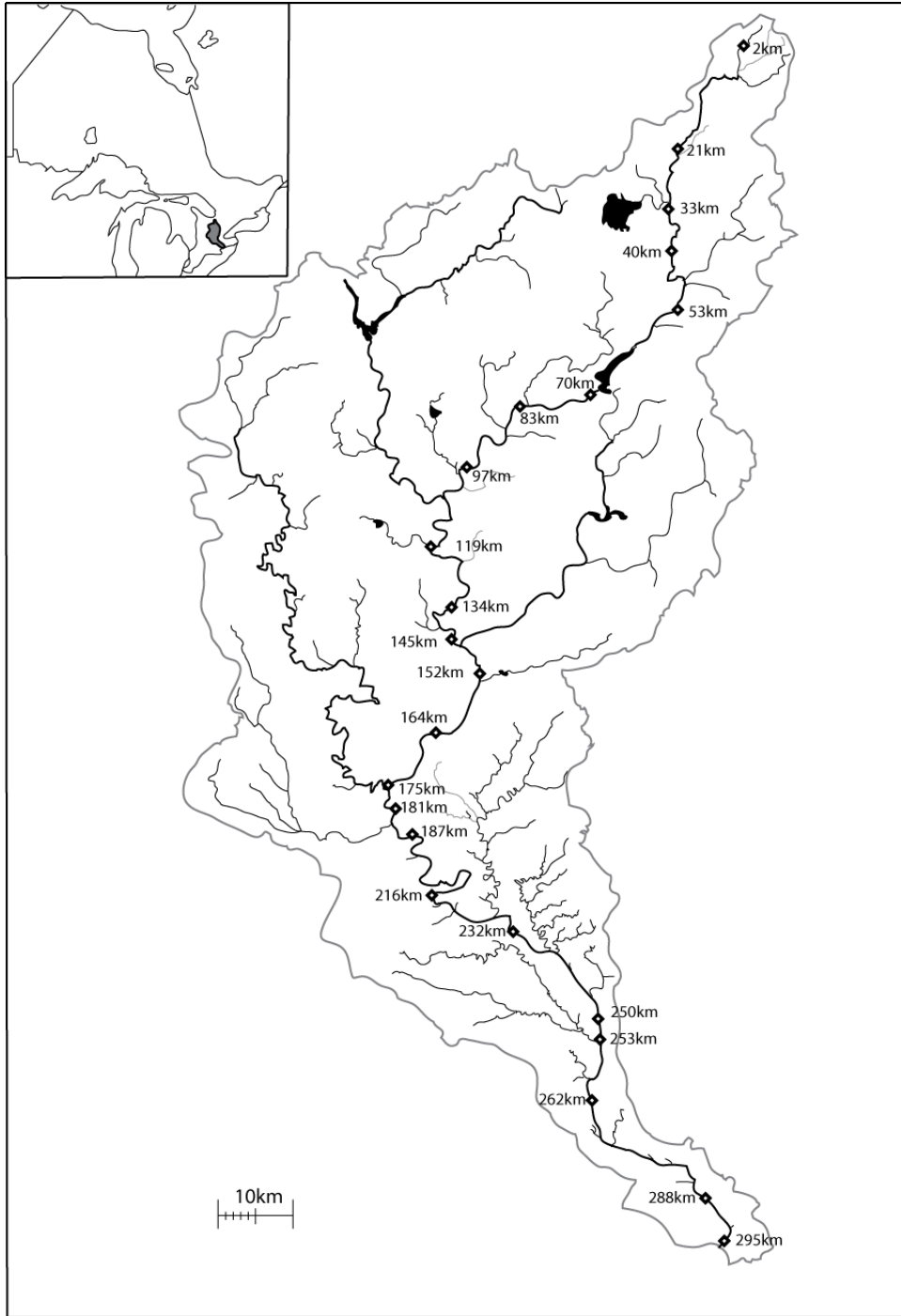
Figure 4.3: Grand River Watershed. Diamonds show sampling locations labelled at river kilometre.
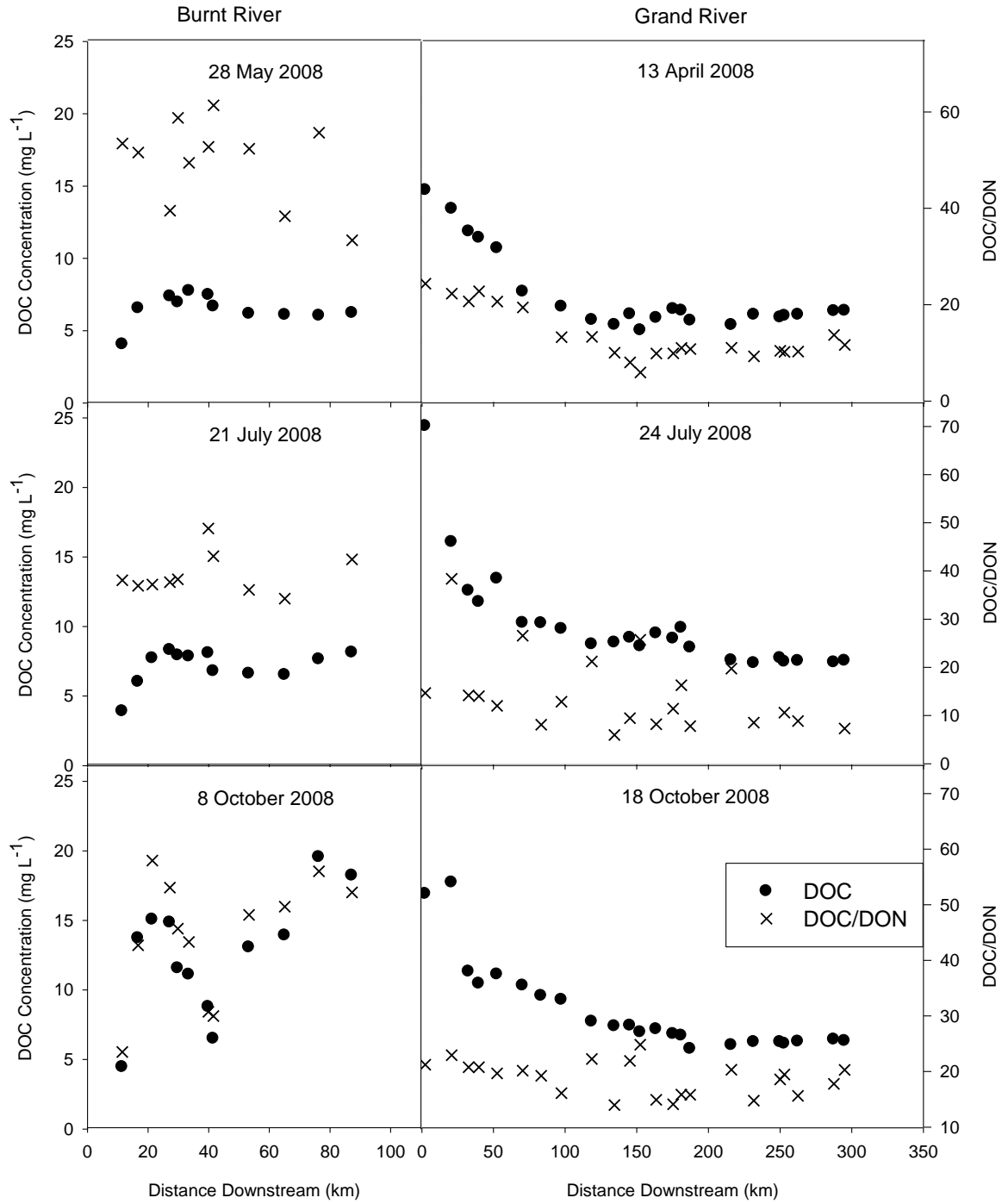
Figure 4.4: Concentrations and ratio of DOC to DON concentrations for 12 sites on the Burnt River and 23 sites along the Grand River
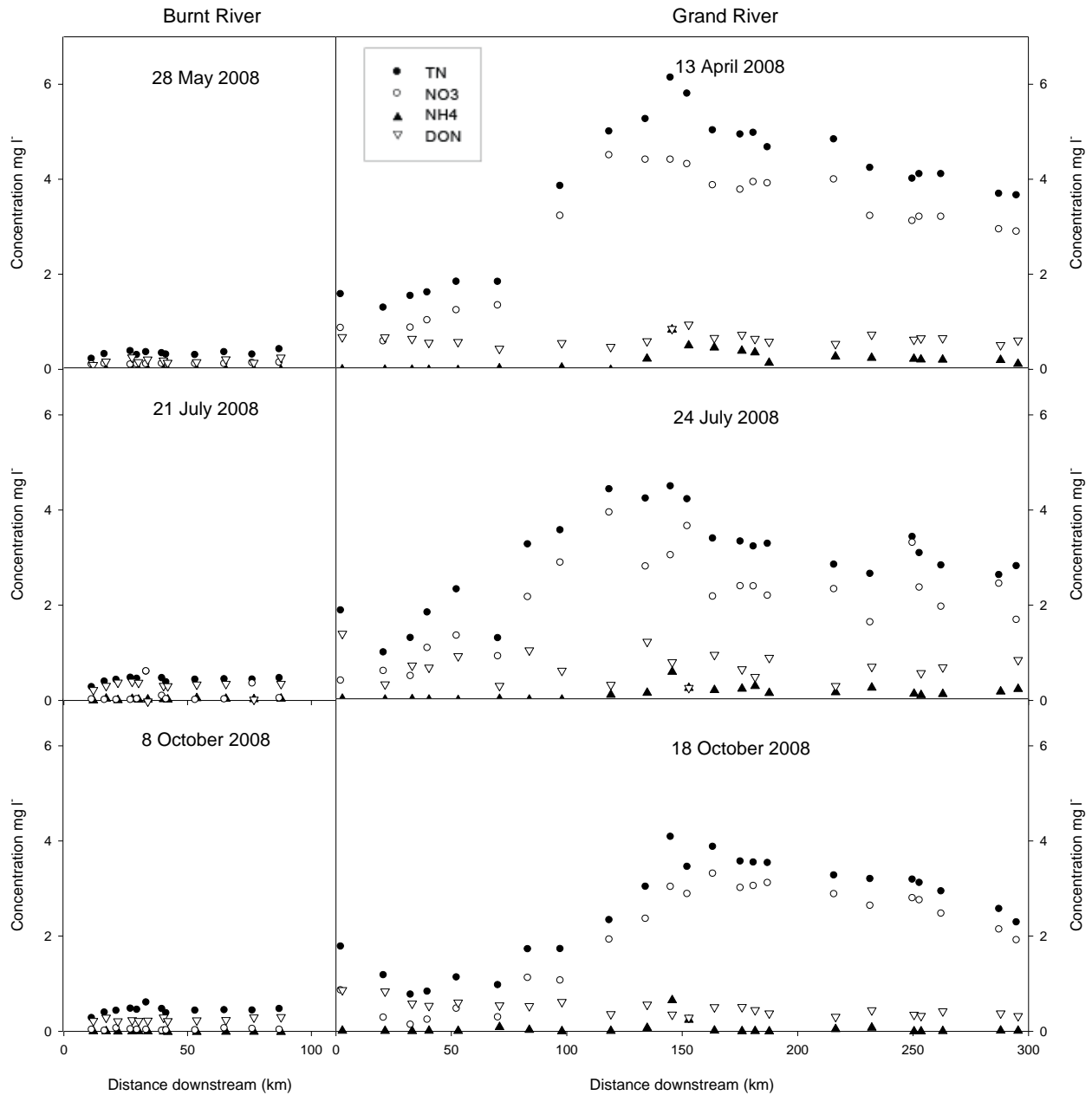
Figure 4.5: Total disolved nitrogen (TDN), nitrate ($NO_3^-$), ammonia/ammonium ($NH_4^+$) and dissolved organic nitrogen (DON) for 12 sites on the Burnt River and 23 sites along the Grand River
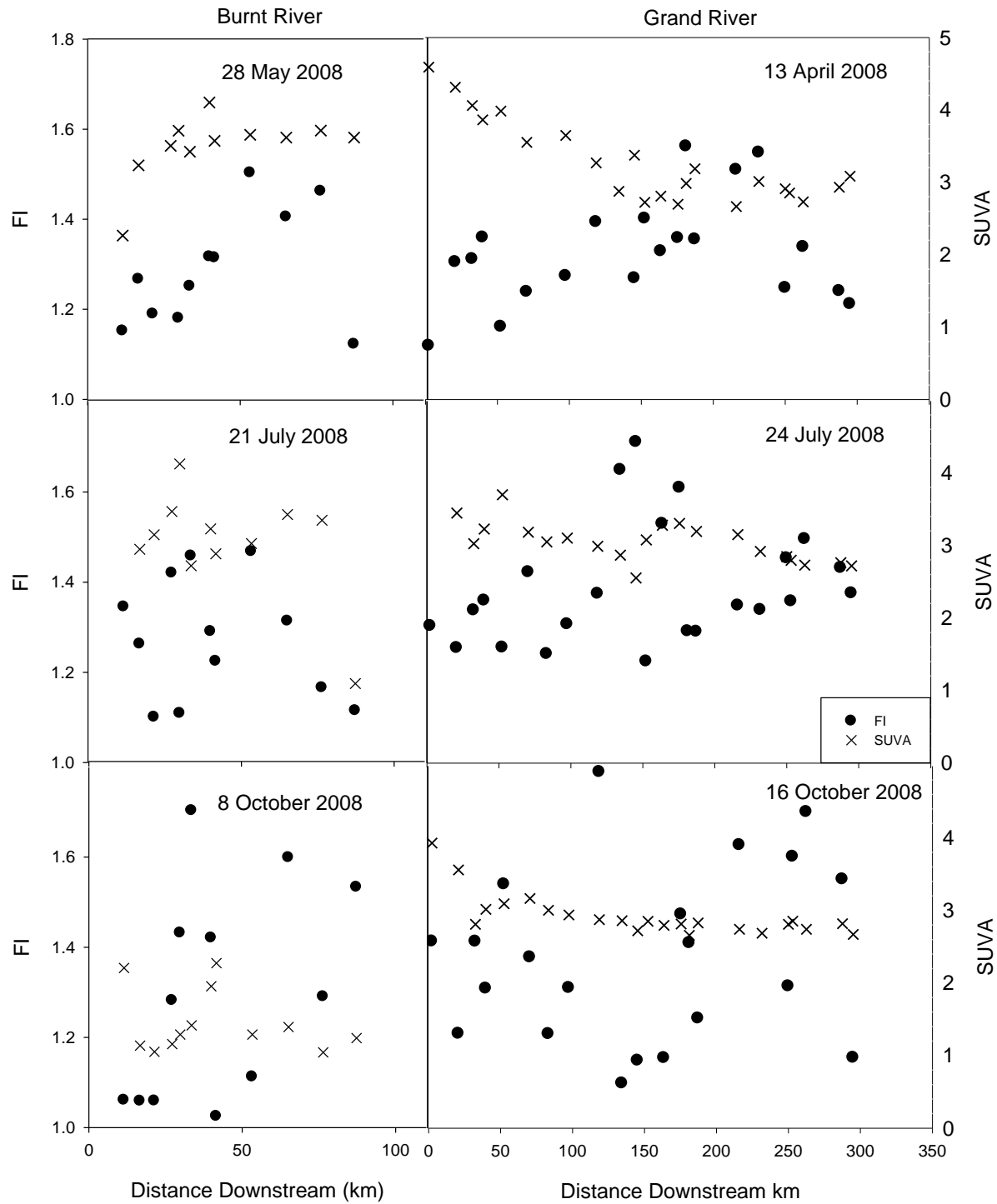
Figure 4.6: Florescence index and SUVA for 12 sites on the Burnt River and 23 sites along the Grand River
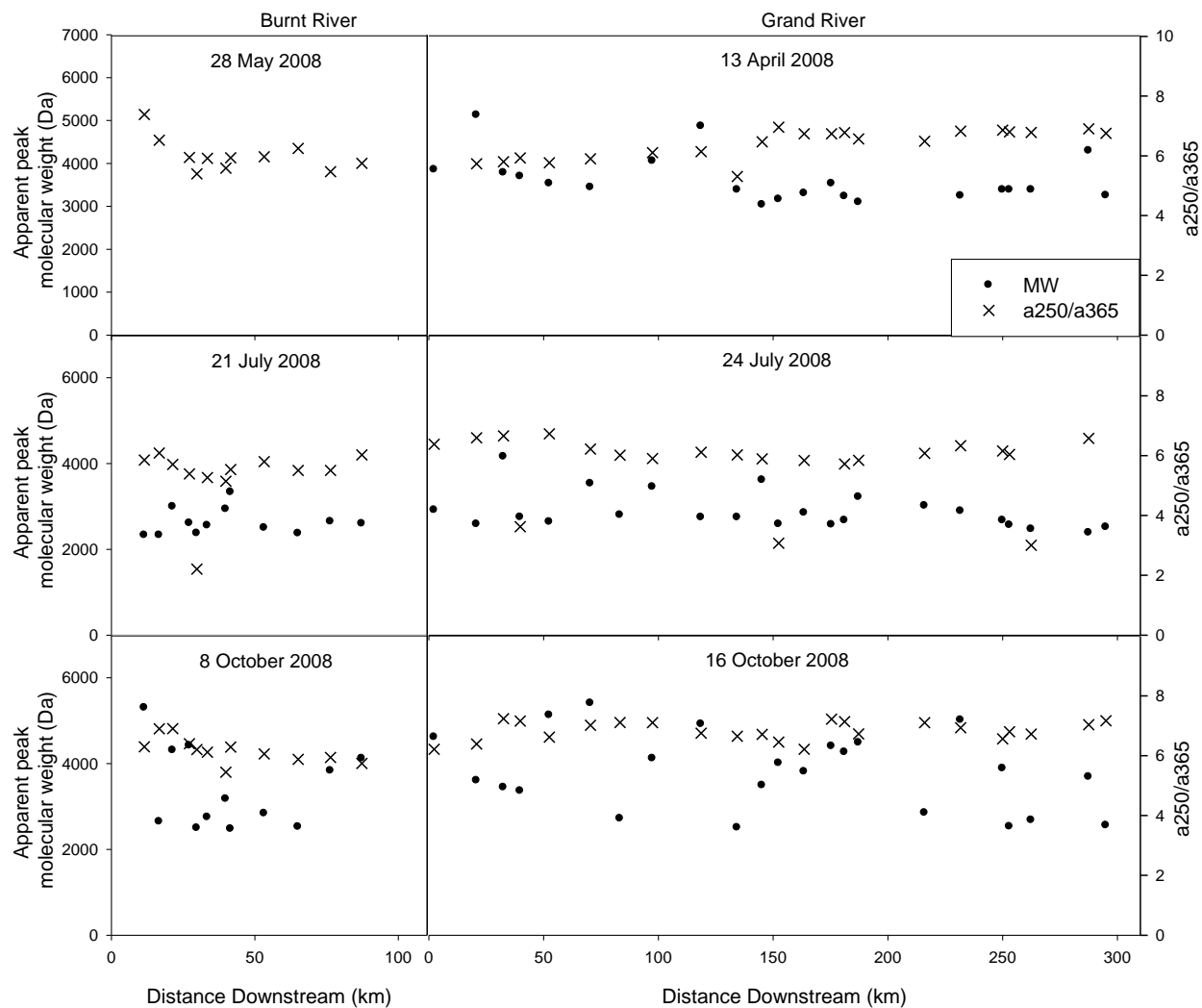
Figure 4.7: Peak apparent molecular weight and absorbance 250 nm/365 nm ratio (an estimate of the proportion of low-molecular-weight to high-molecular-weight DOM) for 12 sites on the Burnt River and 23 sites along the Grand River
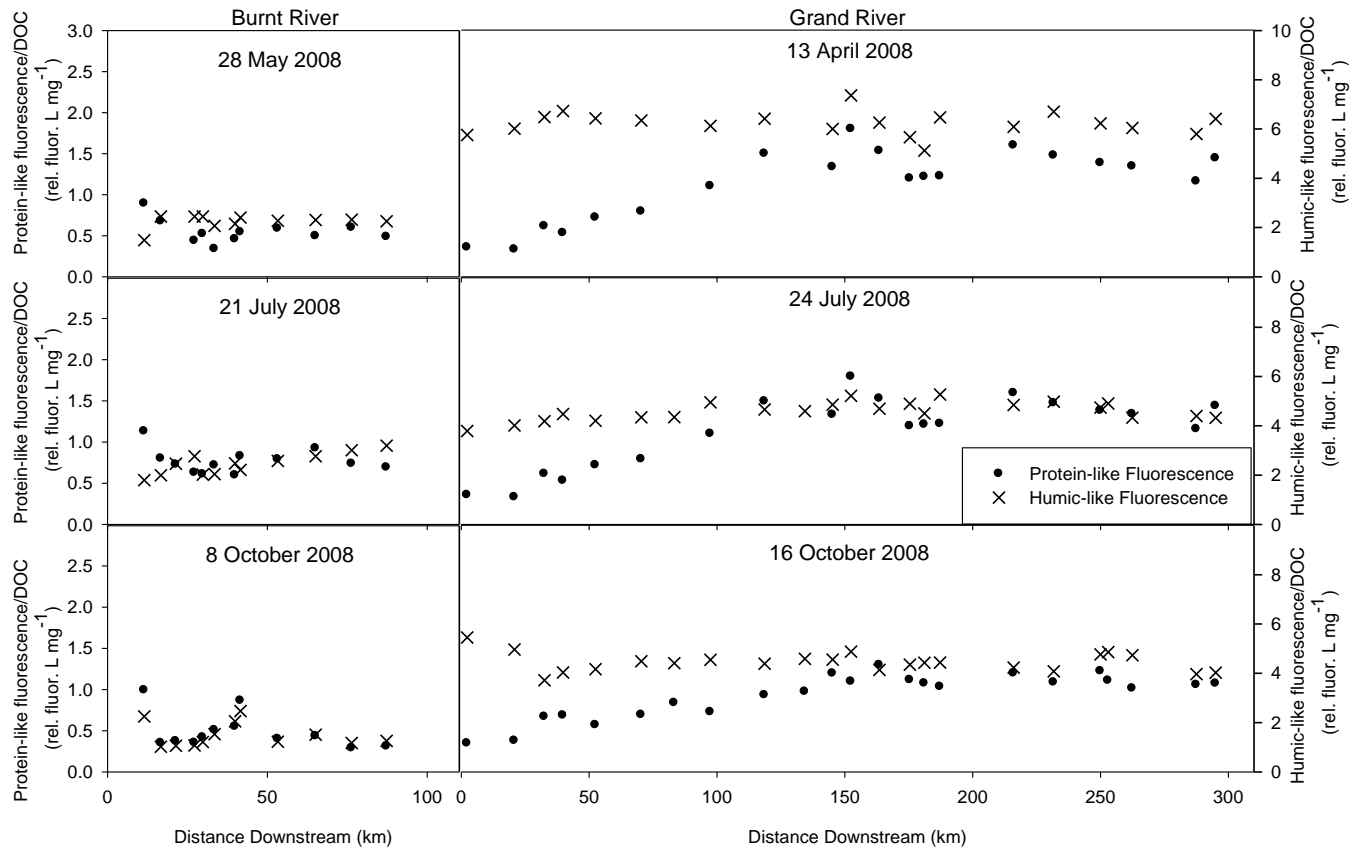
Figure 4.8: protein-like florescence and humic-like fluorescence normalized to DOC
concentration for 12 sites on the Burnt River and 23 sites along the Grand River
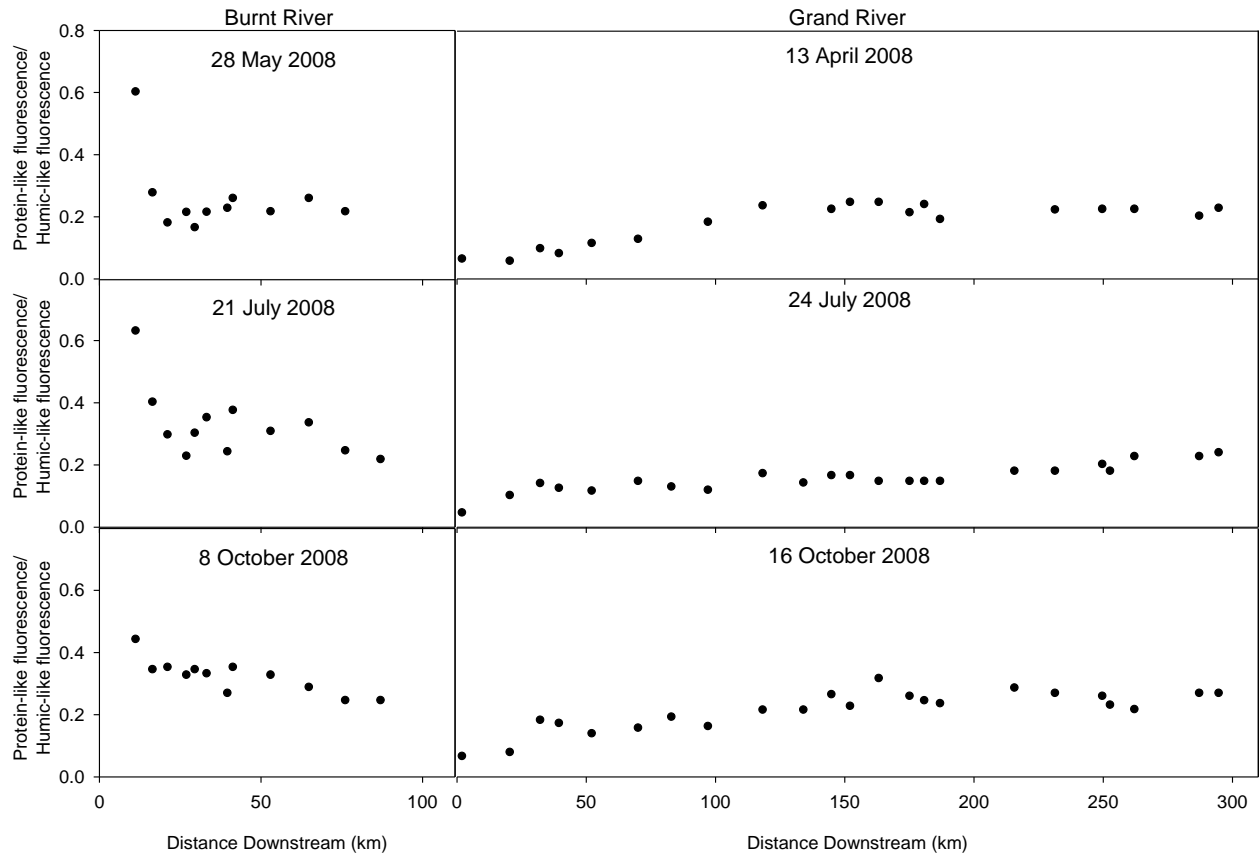
Figure 4.9: the ratio of protein-like florescence to humic-like fluorescence for 12 sites on the Burnt River and 23 sites along the Grand River
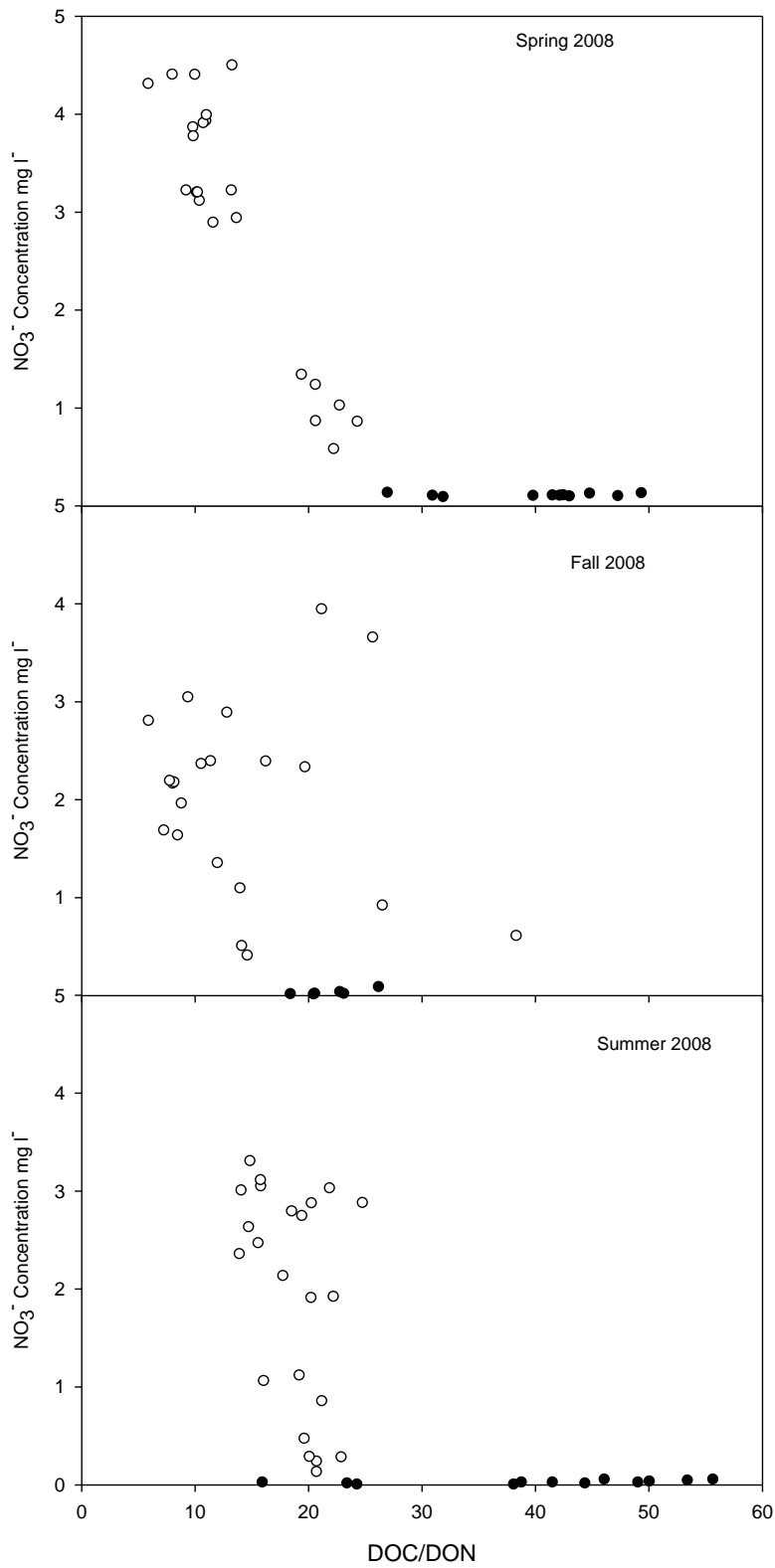
Figure 4.10: Relationship between DOC/DON and nitrate (NO$_3^-$) in the Burnt River (•) and Grand River (o) during 2008 surveys
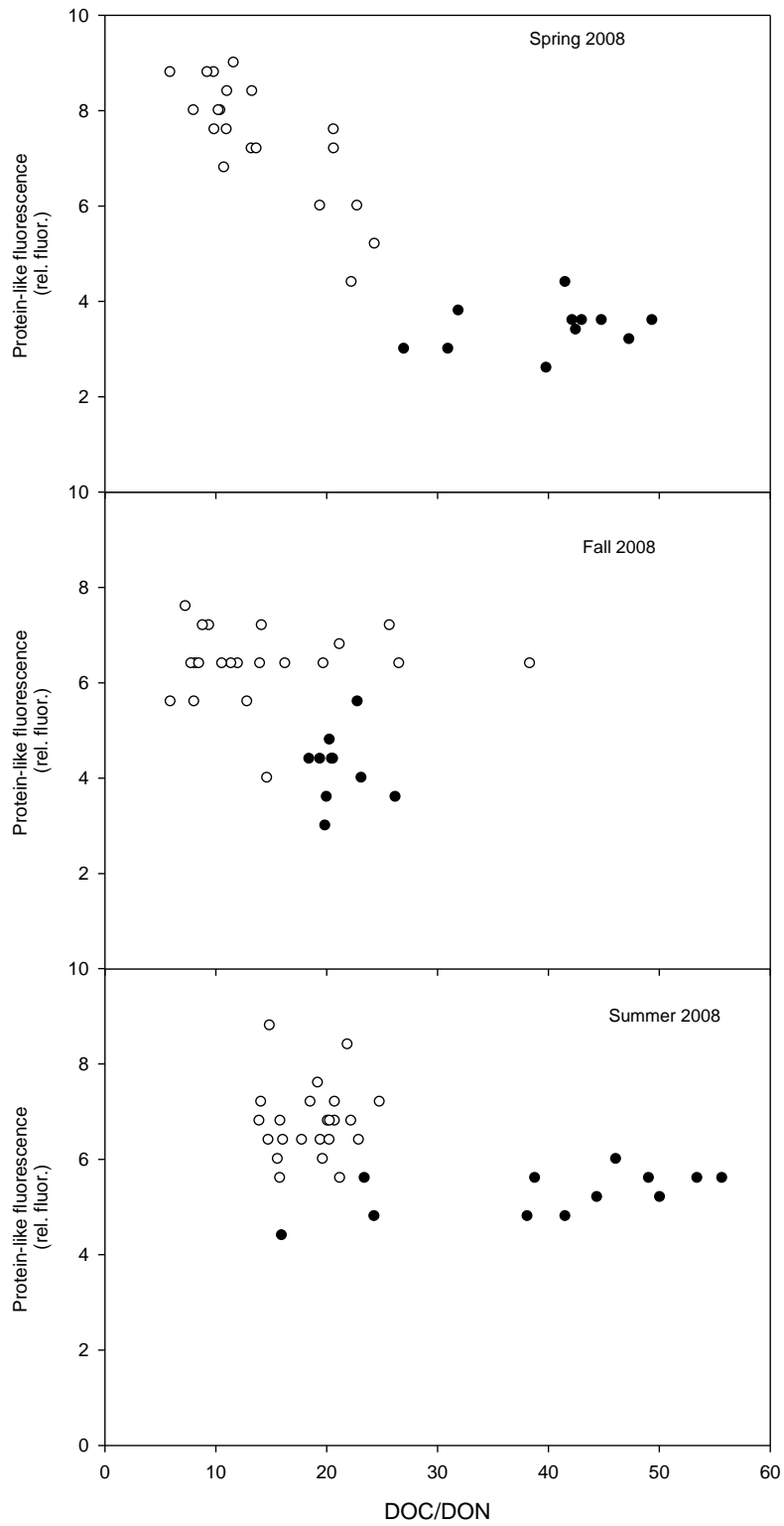
Figure 4.11: Relationship between DOC/DON and protein-like florescence in the Burnt River (•) and Grand River (o) during 2008 surveys
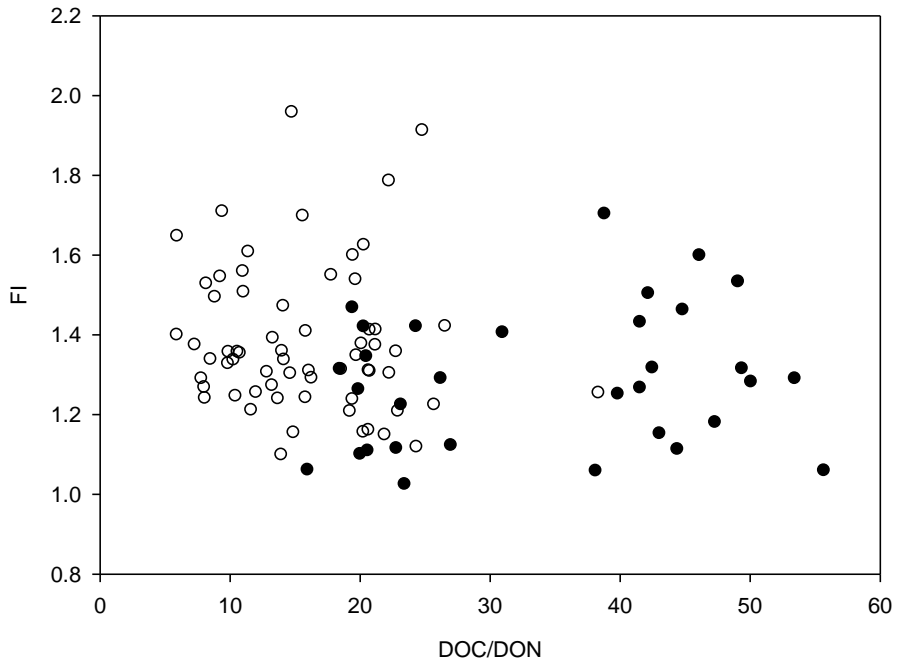
Figure 4.13: DOC/DON and FI in the Burnt River (•) and Grand River during (o) 2008 surveys
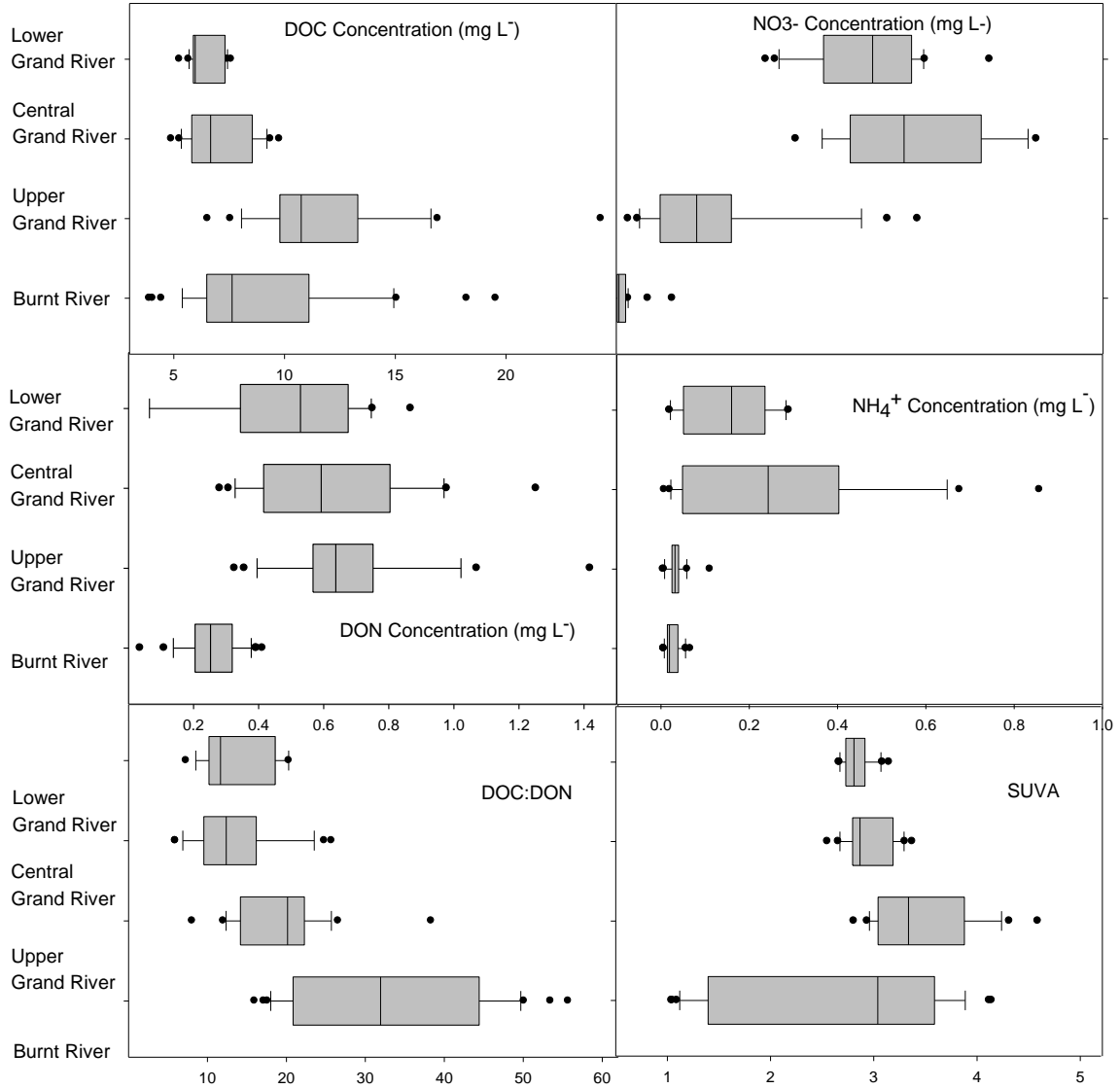
Figure 4.14: Box Plots comparing three Grand River sections: upper, central and lower with the Burnt River in combined spring, summer and fall surveys in 2008 for DOC concentration, $NO_3^-$ concentration, DON concentration, $NH_4^+$ concentration, DOC:DON, and SUVA
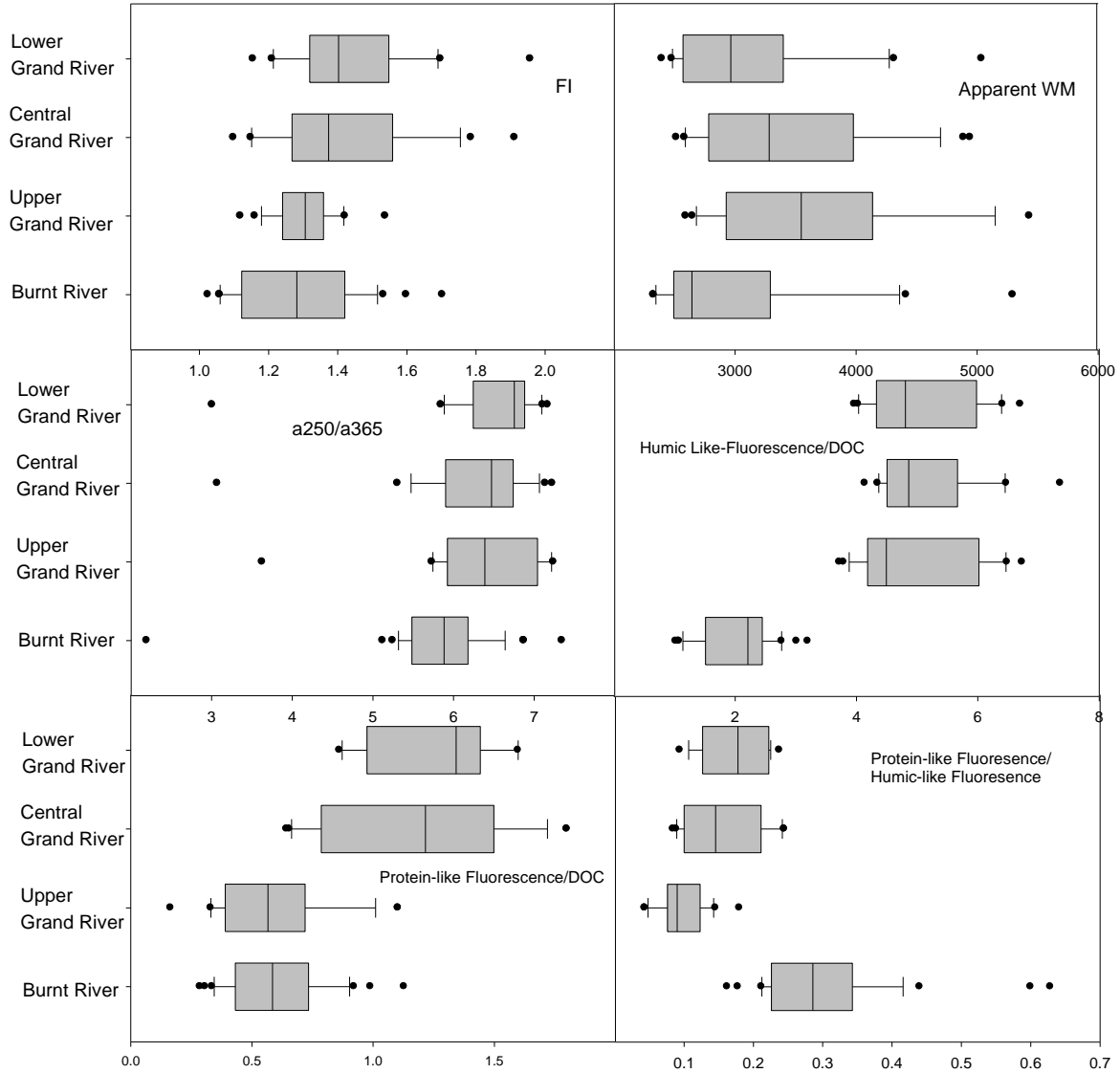
Figure 4.15: Box Plots comparing three Grand River sections: upper, central and lower with the Burnt River in combined spring, summer and fall surveys in 2008 for FI, Apparent WM, a250/a365, Humic-like Fluorescence/DOC, Protein-like Fluorescence/DOC and Protein-like Fluorescence/Humic-like Fluorescence

Table 4.1: Comparison of three Grand River sections: upper, central and lower with the Burnt River during July 2008 for DOC, DOC/DON, DON, SUVA, FI, Apparent WM, a250/a365, Humic-like Fluorescence/DOC, Protein-like Fluorescence/DOC and Protein-like Fluorescence/Humic-like Fluorescence

|  | Burnt River Mean | Range | Upper Grand Mean | Range | Central Grand Mean | Range | Lower Grand Mean | Range |
|---|---|---|---|---|---|---|---|---|
| DOC | 7.1 | 3.9-8.3 | 13.4 | 9.6-24.3 | 8.9 | 8.3-9.8 | 7.4 | 7.2-7.6 |
| DOC/DON | 19.8 | 17-21 | 17.6 | 8-24 | 13.3 | 6-26 | 11.0 | 7-19 |
| DON | 0.3 | 0-0.41 | 0.8 | 0.3-1.4 | 0.7 | 0.3-1.3 | 0.6 | 0.3-0.9 |
| SUVA | 5.0 | 1-26 | 3.2 | 3.0-3.7 | 3.0 | 2.6-3.3 | 2.8 | 2.7-3.1 |
| FI | 1.3 | 1.1-1.47 | 1.3 | 1.2-1.4 | 1.5 | 1.2-1.7 | 1.4 | 1.3-1.5 |
| MW | 2628 | 2327-3332 | 3117 | 2594-4177 | 2888 | 2584-3629 | 2656 | 2396-3026 |
| a250/a365 | 5.3 | 2.2-6.1 | 6.0 | 3.6-6.7 | 5.5 | 3.0-6.1 | 5.7 | 3.0-6.5 |
| Humic | 2.4 | 1.8-3.2 | 4.3 | 3.8-5.0 | 4.8 | 4.5-5.2 | 4.6 | 4.3-5.0 |
| Protein | 0.8 | 0.6-1.1 | 0.6 | 0.3-1.1 | 1.4 | 1.2-1.8 | 1.4 | 1.2-1.6 |
| P/HFlu | 0.33 | 0.22-0.63 | 0.11 | 0.04-0.15 | 0.15 | 0.14-0.17 | 0.20 | 0.18-0.24 |

Table 4.2: Comparison of three Grand River sections: upper, central and lower with the Burnt River during October 2008 for DOC, DOC/DON, DON, SUVA, FI, Apparent WM, a250/a365, Humic-like Fluorescence/DOC, Protein-like Fluorescence/DOC and Protein-like Fluorescence/Humic-like Fluorescence

|  | Burnt River Mean | Range | Upper Grand Mean | Range | Middle Grand Mean | Range | Lower Grand Mean | Range |
|---|---|---|---|---|---|---|---|---|
| DOC | 12.6 | 4.5-19.6 | 11.5 | 8.8-17 | 6.6 | 5.4-7.3 | 5.9 | 5.7-6.1 |
| DOC/DON | 40.1 | 16-56 | 20.1 | 16-23 | 18.0 | 14-25 | 18.1 | 15-20 |
| DON | 0.3 | 0.23-0.32 | 0.7 | 0.31-0.58 | 0.4 | 0.31-0.58 | 0.4 | 0.33-0.46 |
| SUVA | 1.5 | 1.0-2.3 | 3.2 | 2.8-3.9 | 2.8 | 2.7-2.9 | 2.8 | 2.7-2.9 |
| FI | 1.3 | 1.0-1.7 | 1.3 | 1.2-1.5 | 1.4 | 1.1-1.9 | 1.6 | 1.2-2.0 |
| MW | 3404 | 2470-5300 | 4068 | 2730-5430 | 4005 | 2520-4940 | 3330 | 2540-5040 |
| a250/a365 | 6.2 | 5.4-6.9 | 6.9 | 6.2-7.2 | 6.7 | 6.2-7.2 | 6.9 | 6.6-7.2 |
| Humic | 1.5 | 1.0-2.5 | 4.5 | 3.7-5.5 | 4.5 | 4.1-4.9 | 4.4 | 4.0-4.9 |
| Protein | 0.5 | 0.3-1.0 | 0.5 | 0.16-0.63 | 0.7 | 0.64-0.85 | 0.9 | 0.86-1.03 |
| P/HFlu | 0.32 | 0.24-0.44 | 0.08 | 0.04-0.09 | 0.10 | 0.08-0.12 | 0.11 | 0.09-0.13 |
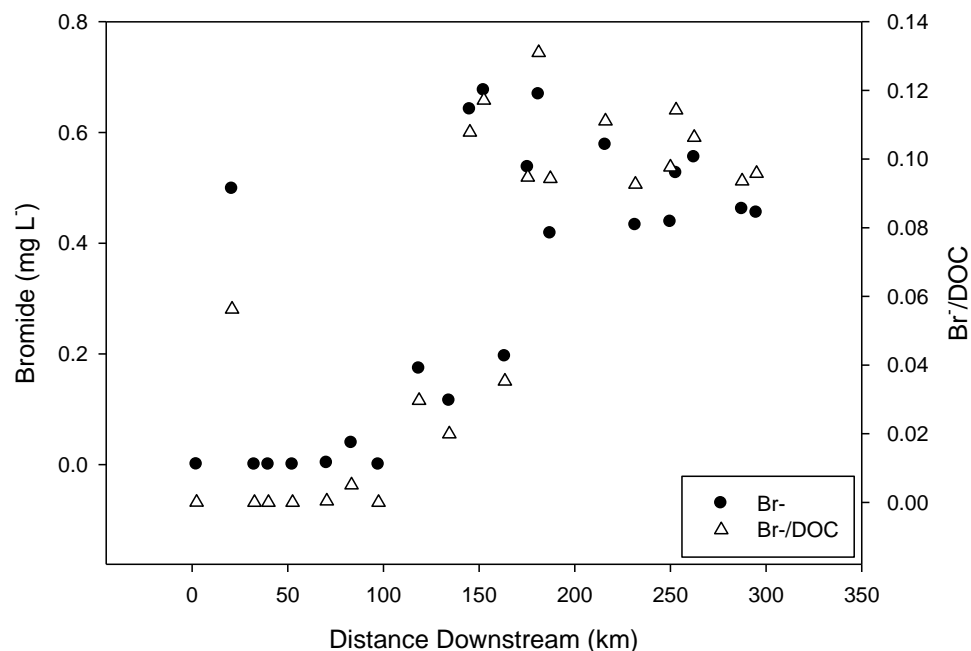
Figure 5.1: Bromide concentrations and Br-/DOC (mg/mg) ratios during Grand River survey on June 26, 2007

Table 5.1: Average THM levels in Brantford, Ohsweken, Kitchener, Waterloo and Dunnville in the raw water, treatment plant and distribution system (Ontario Ministry of the Environment Drinking Water Surveillance Program)

| | THMs (µg/l) | | |
|---|---|---|---|
| | Raw | Treatment plant | Distribution system |
| Brantford | 0.50 | 50.26 | 61.93 |
| Ohsweken | 6.15 | 70.92 | 113.98 |
| Kitchener | 0.50 | 30.19 | 15.56 |
| Waterloo | 0.50 | 6.77 | 9.04 |
| Dunnville | | | 19.70 |

Table 5.2: Average HAA levels in Brantford, Ohsweken, Kitchener, Waterloo and Dunnville in the raw water, treatment plant and distribution system (Ontario Ministry of the Environment Drinking Water Surveillance Program)

|  | HAAs (µg/l) | | |
|---|---|---|---|
|  | Raw | Treatment plant | Distribution system |
| Brantford | 0.58 | 47.01 | 59.16 |
| Ohsweken | 9.86 | 66.17 | 65.62 |
| Kitchener | 0.51 | 45.18 | 13.21 |
| Waterloo | 0.51 | 4.98 | 3.39 |
| Dunnville | 8.26 | 11.39 | 11.49 |

Table 5.3: Average NDMA levels in Brantford, Ohsweken, Kitchener, Waterloo and Dunnville in the raw water, treatment plant and distribution system (Ontario Ministry of the Environment Drinking Water Surveillance Program)

|  | NDMA (ng/l) | | |
|---|---|---|---|
|  | Raw | Treatment plant | Distribution system |
| Brantford | 1.61 | 4.09 | 8.89 |
| Ohsweken | 2.16 | 4.82 | 5.92 |
| Kitchener | 1.92 | 1.91 | 1.86 |
| Waterloo | <1.00 | <1.00 | <1.00 |
| Dunnville | 1.58 | 1.13 | <1.00 |
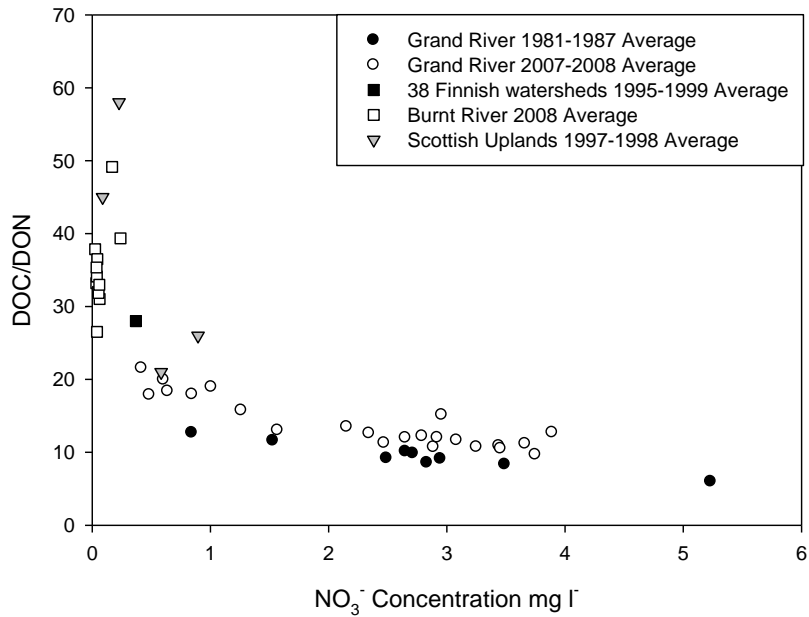
Figure 6.1: Relationship between $NO_3^-$ and DOC/DON: Comparison of PWQMN Site averages in the Grand River watershed from 1981-1987, site averages from Grand River surveys during 2007-2008, site averages from Burnt River Surveys during 2008 and literature values from 38 Finnish watersheds from 1995-1999 (Mattsson et al. 2005) and the Scottish Uplands from 1997-1998 (Chapman et al. 2001)
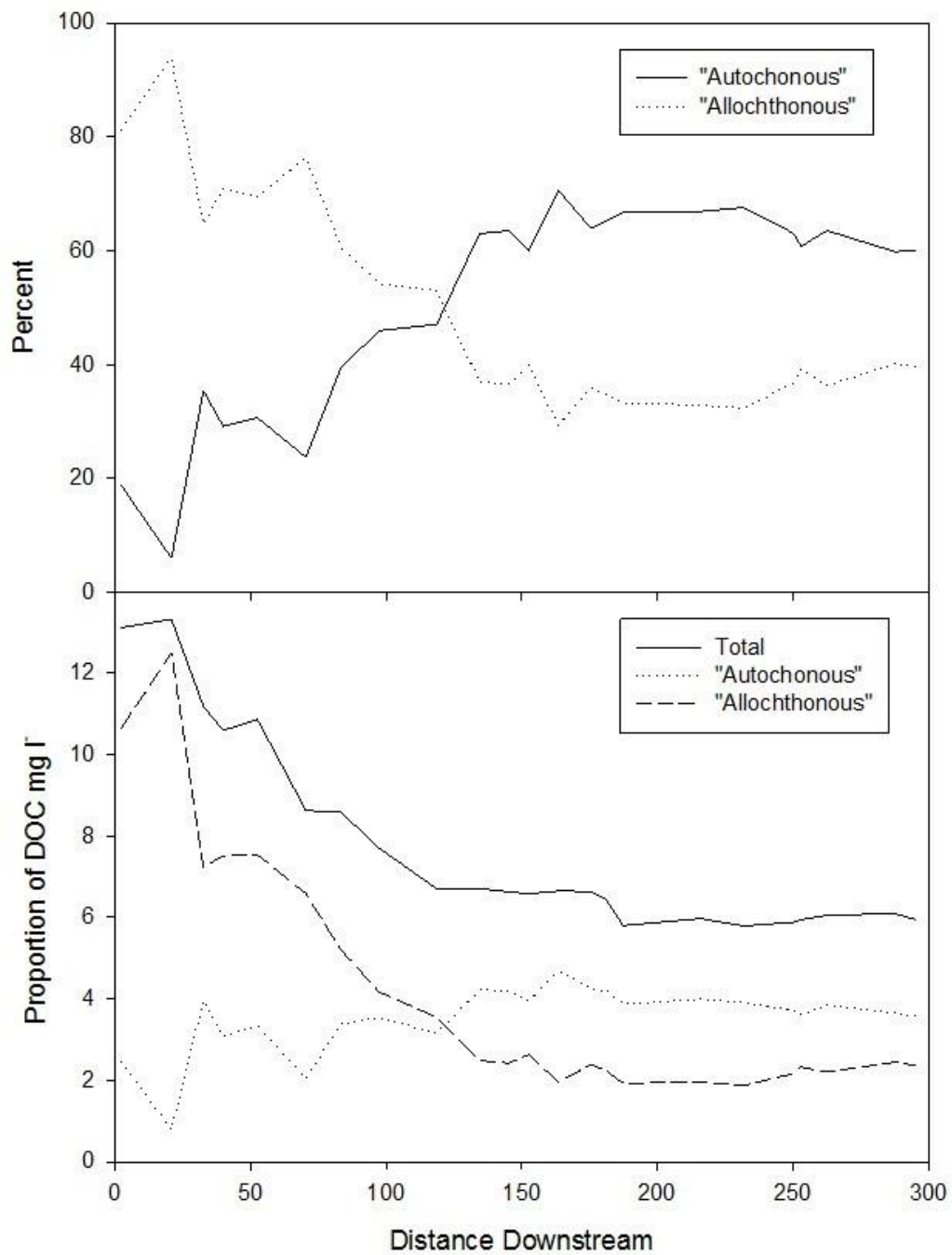
Figure 6.2: Theoretical DOM downstream evolution in the Grand River from 2007-2008 based on a combination of protein-like and humic-like fluorescence and DOC/DON

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