Applications of Fluorescence Spectroscopy for Monitoring Water Quality in an Urban Watershed

By

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Certificate of Approval

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Abstract

In an urban watershed anthropogenic influences can cause detrimental effects on the region's rivers and streams and lead to negative impacts on water quality. Determining the water quality and health of these aquatic ecosystems requires identification of natural and anthropogenic influences and an understanding of the seasonal hydrologic cycle. Dissolved organic carbon (DOC) represents a significant carbon reservoir in all ecosystems and can be used as a means to measure the characteristics and sources of organic matter in aquatic environments. Fluorescence spectroscopy can be used to quantify and characterize a subset of the DOC pool, the colored dissolved organic matter (CDOM), which can absorb and re-emit energy as fluorescence. This study utilizes fluorescence spectroscopy to characterize organic carbon in the Portland, Oregon urban watershed temporally and spatially and to trace the anthropogenic signature found in wastewater effluent associated with treatment plants. Samples were collected from multiple sites within and outside the urban area and from effluent of two different wastewater treatment plants. Several statistical approaches were used to develop a robust model to predict the amount of wastewater present in a stream sample: end-member mixing experiments were conducted to demonstrate the linearity of fluorescence; principal component analysis was used to distinguish sources and characteristics of the organic matter; and a multivariate linear regression model was built using three key fluorescence peaks to characterize the organic matter. The model was validated and predicts the percentage of wastewater in a sample within 80% confidence. The model results can be used to develop in-

situ instrumentation, inform monitoring programs, and develop additional water quality indicators for aquatic systems.

Chapter 1: Thesis Introduction

1.1 Introduction to dissolved organic matter research

As human population growth continues and urban boundaries expand there is an ever-increasing demand for clean healthy aquatic systems for food and drinking water. Over 130,000 km of streams and rivers in the United States are impaired by urbanization. Urbanization has increased the presence of many anthropogenic effects on streams, causing increases in constituents such as oxygen demand, conductivity, suspended solids, ammonium, hydrocarbons, and metals in urban streams (Paul and Meyer, 2008). Anthropogenic inputs into the urban waterways are having adverse effects on water quality, aquatic ecosystems, and habitat.

Many water-quality problems and areas of intense and current scientific research involve dissolved organic matter, both naturally occurring and derived from anthropogenic activities. Taste and odor issues and disinfection by-products associated with the production of clean and healthy drinking water are often associated with dissolved organic matter found in the stream water inflows. Climate and land-use change have resulted and will continue to effect water quality changes in streams that are detrimental, but can be tracked through an examination of the characteristics of dissolved organic matter. Determining the health of a river system requires identification of natural and anthropogenic influences on the biogeochemistry and an understanding of the seasonal hydrological variations. Organic matter is a critical component in certain biogeochemical cycles, and seeking a better understanding of these cycles is necessary for assessing the health of river ecosystems. Specific environmental issues that can be

addressed through an examination of the characteristics of organic matter include determining the importance of algae versus inputs of terrestrial matter in aquatic food webs, and tracking the sources and effects of anthropogenic inputs of sewage and treated wastewater into aquatic environments.

1.1.1 Organic Matter Composition

Organic Matter (OM) is a complex heterogeneous mixture defined as material that is or was once living; capable of decay by biological or chemical processes or the product of decay, or composed of organic compounds. Natural organic matter (NOM) refers to organic matter comprised primarily of decomposing plant and animal material. Typically the NOM pool can be divided into operationally defined fractions of interest; particulate organic carbon (POC) and dissolved organic carbon (DOC). The POC pool is mostly colloidal in nature and defined as the material retained on a .45-micrometer (µm) porosity membrane while the DOC is the organic carbon that passes through the same filter size (Croue et al., 1999). The POC pool generally represents a minor fraction of the total organic carbon (TOC) pool in most aquatic ecosystems. The dissolved organic matter (DOM) fraction typically represents the largest pool of detrital organic carbon that exceeds the organic carbon present in living biomass, and has been considered to be the most important intermediate in the global carbon cycle (Battin et al., 2009). DOM is vital to the ecosystem because it can be highly reactive and is an integral part of microbial food webs, biogeochemical reactions, and UV light attenuation. Dissolved organic carbon (DOC), a main component of the DOM pool, represents a significant global carbon reservoir needed in all ecosystems and can be used as a water quality indicator.

Humic substances make up approximately 60-70% of the total organic carbon in soils and 40-60% of the dissolved organic carbon pool in natural waters (Beck et al., 1993). Humic materials are typically of high molecular weight and can be aromatic (carbon ring molecular structures) or aliphatic (straight-chain carbon molecular structures), and are rich in oxygen-containing functional groups (Stevenson, 1982). Formation of humic substances is one of the least understood aspects of humus chemistry. Several potential pathways exist for the formation of humus material; depending on the sources of NOM usually determines the pathway of formation. Humic substances can be formed by- plant decomposition and transformation by microorganisms which in turn form sugars, polyphenols, amino acids, and decomposed lignin products, or through the breakdown of quinones (Aiken et al., 1992, Stevenson, 1982).

Regardless of the path of formation humic substances are primarily composed of three distinct components; humic acids, fulvic acids, and humins, distinguishable from each other by chemical and physical characteristics (Stevenson, 1982). Humic acids are thought to be dark-brown to grey-black complex aromatic macromolecules with amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups (Mobed et al., 1996). The structure and composition of humic acids varies according to their sources and methods of extraction. The humic acid components of NOM are typically the largest fraction in molecular weight with longer chain fatty acid products and therefore, a higher hydrophobicity than the other components (Leenheer and Croue, 2003). NOM remains relatively elusive in its chemical composition due to the complexity and ever-changing sources and stages of degradation such that it is difficult to designate a specific molecular structure that can describe all humic acids. Fulvic acids, the second major component of NOM, tend to be moderate in

molecular weight and light-yellow to yellow-brown colored organic acids. Aquatic fulvic acids with significant aromatic content derived from plant litter and soils contribute approximately 25-30% of the total carbon pool (Malcolm, 1990). Another primary source of fulvic acids are microbially derived with lower aromaticity and represent approximately 12-17% of the total carbon pool (McKnight et al., 1991). Similar to humic acids, the molecular composition of fulvic acids is highly variable and a function of the various precursor organic materials. In general, fulvic acids are more soluble, lower in oxygen and carbon content, and lighter in molecular weight than humic acids (Stevenson, 1982). The content and characteristics of a specific NOM sample is highly dependent on its precursor material which is highly variable in nature.

1.1.2 Aquatic Organic Matter

Dissolved organic matter (DOM) can be leached out of soils and decaying plants and animals, produced by algae in the water column, and augmented by anthropogenic inputs (e.g. wastewater discharge and agriculture production). For aquatic systems, sources of DOM are commonly categorized as: (1) allochthonous— material entering water from terrestrial, wetland, and littoral zones, and (2) autochthonous— material derived from biota (e.g. algae, bacteria, and macrophytes) within the aquatic ecosystem from both the pelagic and benthic zones (Aiken and Cotsaris, 1995, Reckhow et al., 2008). The concentration and chemical quality of DOM in aquatic systems that originates from terrestrial-derived material can be an indicator of watershed-scale biogeochemical processes (Hood et al., 2005). Varying sources of DOM in aquatic systems contain a variety of classes of organic compounds with differing reactivity and ecological effects (Maie et al., 2006). A prime example of this is the role of the aromatic carbon fraction of DOM, typically associated with humic and fulvic acids, that are mostly responsible

for attenuating harmful UV light in aquatic systems (Jaffé et al., 2008). In addition to varying sources of DOM, the interactions of the same DOM pool can vary depending on where in a given ecosystem it occurs. For example, DOM at the sediment-water interface tend to form complexes with trace metals and are involved in their transport or retention, while DOM in the photic-zone of the water column tends to have an impact on light attenuation. To further complicate the role of aquatic DOM, the quantity and quality are dynamic and can be altered by hydrologic events and seasonal variations (Lu et al., 2003). Thus, understanding the quality and quantity of aquatic DOM and how it is transformed and transported through a system has become a key area of scientific research. Scientists are looking to develop tools to assess aquatic ecosystem functions, biogeochemistry cycling, food web dynamics, watershed management, chemical pollutants, carbon budgets, and overall health of these ecosystems.

1.2 Introduction to Fluorescence Spectroscopy

Molecular characterization and quantification of aquatic DOM can be measured with many high resolution molecular measurements. One common first step in high resolution analysis is to fractionate the complex mixture into distinct polarity fractions (hydrophobic/hydrophilic) using XAD-8 isolation methods (Aiken et al., 1992). After separation, measurements can be made to identify molecular compounds found in each fraction with methods such as, elemental analysis using ^{13}C NMR spectroscopy, multistage mass spectrometry coupled with electrospray ionization, pyrolysis gas chromatography/mass spectrometry, and high pressure size exclusion chromatography (Aiken et al., 1992, Hernes et al., 2009, Leenheer and Croue, 2003, Weishaar et al., 2003). These techniques can provide qualitative information for identifying specific compounds and biopolymers found in complex

mixtures of DOM but have several limitations. All of the high-resolution analyses require expensive, sophisticated instrumentation and significant sample preparation. The analyses can be cost prohibitive, for example charging approximately $10,000 per sample for the more sophisticated technologies (Croue et al., 1999). The research limitations have motivated scientists to find an inexpensive, simple, effective method to analyze both quantitative and qualitative properties of complex DOM. Fluorescence spectroscopy can rapidly measure large amounts of samples in near real-time with rapid and precise characterization of DOM (Fellman et al., 2009).

Chromophoric dissolved organic matter (CDOM) is the operationally defined component of the total DOM that absorbs light over a broad range of visible and UV wavelengths (Coble, 1996). CDOM is a highly variable component of the DOC pool, and can affect the optical properties of natural waters by directly impacting the magnitude and spectral distribution of light in the water column and in turn modifying the water hues (Chen et al., 2003, Del Vecchio and Blough, 2004, Vodacek et al., 1997). A component of CDOM can be detected by measuring the fluorescence emission at blue-green wavelengths, with an optimum wavelength centered at 420-450nm (Breves and Reuter, 2000, Coble, 1996, De Souza Sierra and Donard, 1991). The use of absorbance and fluorescence spectroscopy as proxies for DOC concentration has been recognized for many years in the field of oceanography (Coble, 2007), and more recently a number of studies have shown that absorption coefficients (e.g. A_{254}, A_{412}, A_{350}, A_{440},) and fluorescing dissolved organic matter (FDOM)(ex. 370 nm/em. 450 nm) are good predictors of DOC concentration in surface waters (Belzile et al., 2006, Bergamaschi et al., 2000, Downing et al., 2009, Saraceno et al., 2009). However, the fluorescence-response of a natural

water sample to light excitation is variable depending on precursor materials, molecular composition, and the stage of organic matter decomposition (Downing et al., 2009, McKnight et al., 2001).

1.2.1 Fluorescence Spectroscopy

While absorbance spectroscopy measures the amount of light transmitted through a sample (i.e. the ability of dissolved substances in the water to absorb light at specified wavelengths), fluorescence spectroscopy measures the light that is re-emitted by a given wavelength. The process that occurs between absorption and emission of light are best described by Jablonski's diagram (Lakowicz, 2006). Each fluorophore has a specific affinity for light absorption at a specific wavelength and is then excited to some higher vibration level followed by photon emission at a lower vibration level or wavelength (Stokes Shift) and thus can be measured using optical instrumentation (Figure 1.1).

Fluorescence measurements in this thesis were conducted using a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer. The light source is a 150-W ozone free xenon arc-lamp. The light from the lamp is collected by a diamond-turned elliptical mirror, and then is focused on the entrance slits of the excitation monochromator. A grating mechanism is used to disperse the incident light and by rotating the gratings a spectrum of emission wavelengths is achieved. Both the excitation and emission monochromators have adjustable slit widths that control the amount of light emitted which determines the degree of sample interrogation achieved. The Fluoromax-4 contains two detectors; the signal and reference detectors. The signal detector is a photomultiplier tube (PMT) for photon counting and the reference detector monitors the

xenon lamp in order to correct for wavelength and time dependent output of the lamp. Excitation wavelengths can be incrementally increased starting from 240nm intervals of 10nm and therefore identify multiple excitation/emission characteristics of a natural sample.

Fluorescence spectroscopy was used in a wide range of problems in the chemical and biological sciences. Recent scientific explorations have expanded the use of fluorescence spectroscopy to include ocean and freshwater investigations of CDOM. The optical properties of CDOM provide information on both the amount of material present and the chemical properties of the bulk sample, which can undergo changes due to chemical, biological, and physical processes. Fluorescence measurements of the CDOM fraction of aquatic DOM are often measured across a range of excitation and emission wavelengths creating a complex 3-dimensional excitation-emission matrices (EEMs,) which are detailed landscapes of distinctive peaks that can provide indications of sources, behaviors, and biogeochemical cycling of the material (Coble, 2007, Hudson et al., 2007a, Stedmon and Bro, 2008, Murphy et al., 2010). Many key excitation-emission pairings have been identified that represent specific characteristics and sources of NOM and have been described throughout the DOM literature (Figure 1.2 and Table 1.1).

DOM absorbance measurements have elucidated information on molecular structure and composition. For example, specific UV-absorbance at 254 nm ($SUVA_{254}$) normalized by DOC concentration, has been shown to correlate to aromatic content (Weishaar et al., 2003). Similarly, the spectral slope, which is the slope of the absorbance over the 275-295 nm wavelength range ($S_{275-295}$), has been shown to correlate to DOM aromatic content and

molecular weight. For example, lower $S_{275-295}$ is associated with higher aromatic content and higher molecular weight. Spectral slope has also been shown to change upon UV-irradiation (Helms et al., 2008, Spencer et al., 2009) as a result of molecular susceptibility to photodegradation. Similarly, fluorescence data has been shown to provide information about DOM character and origin. The fluorescence index (FI), calculated as the ratio of emissions from 470 nm to 520 nm at excitation of 370 nm, has been widely used to indicate relative contributions of algal (autochthonous) versus terrestrial (allochthonous) derived DOM. Higher FI is associated with algal derived material which has lower aromatic content and lower molecular weight, while lower FI is indicative of more highly processed, terrestrial derived material that has greater aromatic content and higher molecular weight (Jaffé et al., 2008, McKnight et al., 2001). Qualitative information can also be derived from the identification of fluorescence peaks produced from the full EEMs spectra that have been linked to different DOM pools such as humic and fulvic acids, protein-like material and phytoplankton derived material (Coble, 2007, Hudson et al., 2007a, Stedmon et al., 2003) (Table 1.1). Differences in the chemical make-up of the DOM pool can then be linked to changes in DOM reactivity and may be used to infer DOM sources (Jaffé et al., 2008, McKnight et al., 2001, Spencer et al., 2007, Stedmon et al., 2003).

1.2.2 Fluorescence Measurement Processing

In order to use fluorescence measurements quantitatively and compare measurements from different instruments a series of post-processing steps are required. Fluorescence data is typically retrieved from the instrument in raw units (photon counts). Typically these units are converted to either a quinine sulfate factor computed from a series of dilution runs (Coble et

al., 1993, Hoge et al., 1993) or to the integrated area under a clean water Raman scan (Stedmon and Bro, 2008). Samples need to be corrected for an Inner Filter Effect (IFE) that can occur from optically dense samples. Several methods exist for correcting for this optical phenomena but the most commonly used and agreed upon method is the Lacowicz method (Lakowicz, 2006). The corrected fluorescence intensity is given by,

$$F_{corr} = F_{obs} \text{ antilog } ((OD_{ex} + OD_{em})/2) \quad (1)$$

Where OD represents the optical density of the sample at both excitation and emission wavelengths (Lakowicz, 2006). Most fluorescence spectroscopy instruments come with a manufacturer correction files to correct for wavelength dependencies of each optical component for both the signal and reference detectors. Another important step in EEM post processing is to subtract a pure water blank to separate out the background fluorescence associated with a clean water sample (Murphy et al., 2010). The DOM fluorescence research scientists agreed to set protocols for EEM post-processing and have determined the ideal series of procedures for the execution of each step (Figure 1.3).

The application of multivariate statistical analyses, such as Parallel Factor Analysis (PARAFAC), can provide further insight into DOM quality, source, and processing (Cory and McKnight, 2005, Jaffé et al., 2008, Kraus et al., 2010). PARAFAC is a multiway decomposition method that decomposes the fluorescence signal of DOM into unique fluorescent groups whose abundance can be related to DOM precursor materials (Stedmon et al., 2003). It can further identify different classes of OM which make up the entire EEMs spectra, and the

relative proportions of these components can reveal both quantitative and qualitative differences between samples (Stedmon and Bro, 2008).

1.2.3 In-situ Sensors Using Fluorescence Spectroscopy

The in-situ fluorometer has been used on many occasions and in many different environments to provide a high resolution proxy for DOC concentration and in some cases, other related biogeochemical variables such as trihalomethane (THM) precursors and methyl mercury (MeHg) concentrations. There is no a priori assumption, however, that FDOM will directly correlate with DOC concentration in every environment, especially those in which the DOC pool is relatively fresh and labile such as in highly productive environments (Spencer et al., 2009). This is because the optical properties of DOM is a function of its chemical composition; if the fraction of the DOM pool that fluoresces changes in a non-linear fashion with DOC concentration, then the relationship between FDOM and DOC concentration may not hold up. For example, absorbance per mole of DOC ($SUVA_{254}$) has been shown to be site and time dependent, demonstrating that changes in DOC quality affects the relationship between absorbance and DOC concentration (Weishaar et al., 2003). This should also apply to fluorescence measurements. For this reason, optical properties can't provide a universal proxy for DOC concentrations; however, based on the success of previous studies a relation between optical proxies and DOC concentrations can be verified for specific locations and thus provide a robust proxy for DOC concentration on a site-specific basis (Kraus et al., 2010).

Recent work applied to an agricultural watershed demonstrated that the in-situ FDOM sensor accurately predicted DOC concentrations throughout a precipitation event, where DOC

concentration increased from 2 to 10 mg/L within hours of the start of precipitation (Saraceno et al., 2009). Several recent studies from a range of environments including wetlands, forested watersheds, and tidal marshes produced predictive relationships between WETLabs CDOM fluorometer data and laboratory determined DOC concentration (Downing et al., 2009).

1.3 Study Overview

Research has shown that urban watersheds are increasingly degraded by development and human population growth (Paul and Meyer, 2008). The growth in these urban regions and the associated changes in landscape have increased the stresses to freshwater ecosystems and the water-quality. Changes in landscape have caused encroachment of urban land into riparian areas thereby decreasing canopy cover and increasing solar radiation to the streams. Expansion to human populations has increased the amount of industrial and human waste, which has created higher demands on the wastewater treatment plants. Wastewater effluent, personal care products, and increased agriculture pesticides have added to the deterioration of water quality found in urban streams (Waite et al., 2008). Population growth increases the demand for clean, healthy drinking water from the same impaired streams.

The Willamette River Basin and surrounding area includes 35,000 km^2 in northwest Oregon and southwestern Washington. The drainage area in the Willamette Valley combines natural tributaries, complex networks of canals and agricultural areas, and sewer piping in the cities. Dams and reservoirs regulate most of the rivers in the area, which supply drinking water, power generation, and irrigation to different parts of the region (Carpenter and McGhee, 2009, Waite et al., 2008).

1.3.1 Specific areas of research interest

On the west side of Portland Oregon metropolitan area, the Tualatin River Basin contains approximately 500,000 people in the urbanized lower reaches of the river. Clean Water Service is the primary wastewater and stormwater management utility for the urban areas of the Tualatin River Basin. Clean Water Services treats approximately 60 million gallons of wastewater per day through the operation of four wastewater treatment plants (WWTPs) that are among the most advanced in the Nation at removing phosphorus and other constituents from wastewater. Even with the advanced treatment processes during low flow periods, the Tualatin River, can contain up to 40% treated wastewater (Bonn, 2009). Anthropogenic inputs into stream systems are having adverse effects on water quality, aquatic ecosystems, and habitat (Paul and Meyer, 2008). The tributaries of the Tualatin River have water-quality problems associated with periodic low dissolved oxygen levels that can drop below the state's total maximum daily levels (TMDL) which can pose a potential health issue for resident fish. High rates of oxygen demand that can occur in shallow streams and tributaries are attributed to organic matter that settles at the sediment-water interface in streams. The OM consumes substantial amounts of oxygen as it decomposes resulting in lower dissolved oxygen concentrations in those streams. To improve oxygen conditions in the Tualatin River tributaries it is critical to identify, quantify, and better manage the sources of organic matter and fine organic-rich sediment that deposit in the streams as well as the amount of organic matter residing in and moving through those streams.

The Clackamas River in northwestern Oregon is a valued resource to the region, providing drinking water for about 380,000 people. The Clackamas River begins on the slopes of

Ollalie Butte near Mt. Hood, a high Cascade Range volcano, and flows 82 miles from its headwaters at about 6,000 feet elevation down to its confluence with the Willamette River southeast of Portland. The watershed drains an area of nearly 940 square miles, winding through forests, mountain meadows, farmlands, industrial areas, and densely populated urban areas. Oregonians depend on the Clackamas River for their supply of drinking water, hydroelectric power, and outdoor recreation opportunities. The watershed supports a rich variety of native plants, diverse forests, fish, and wildlife habitats. During drinking-water treatment (e.g. chlorination/bromination) a portion of the natural organic matter (NOM) in water reacts to form toxic disinfection by-products (DBPs) which have been shown to be carcinogenic and mutagenic (Leenheer and Croue, 2003). The amounts of DBPs that form during treatment are both a function of the amount and character of the organic matter pool. The specific pool of organic matter that reacts to form DBPs is commonly referred to as DBP precursors.

1.3.2 Study Rationale

Many urban water quality problems have a common link, organic matter. Tracking sources and examining characteristics of organic matter are necessary steps towards monitoring related impacts on water quality in streams and finished drinking water. Applications of fluorescence spectroscopy enable the rapid and precise characterization of DOM and can trace the changes in quality and quantity in these urban streams. Examining urban water quality problems associated with organic matter and the information obtained from fluorescence spectroscopy can lead to improvements in water quality monitoring and can inform water managers to best management practices.

Chapter 1 Figures

Figure 1.1: Modified from Jablonski's diagram, the graph illustrates the process that occurs between absorption and emission of light. Each fluorophore has a specific affinity for light absorption at a specific wavelength and is then excited to some higher excited state level followed by photon emission at a lower vibration level or wavelength (Stokes Shift) and thus can be measured using optical instrumentation.

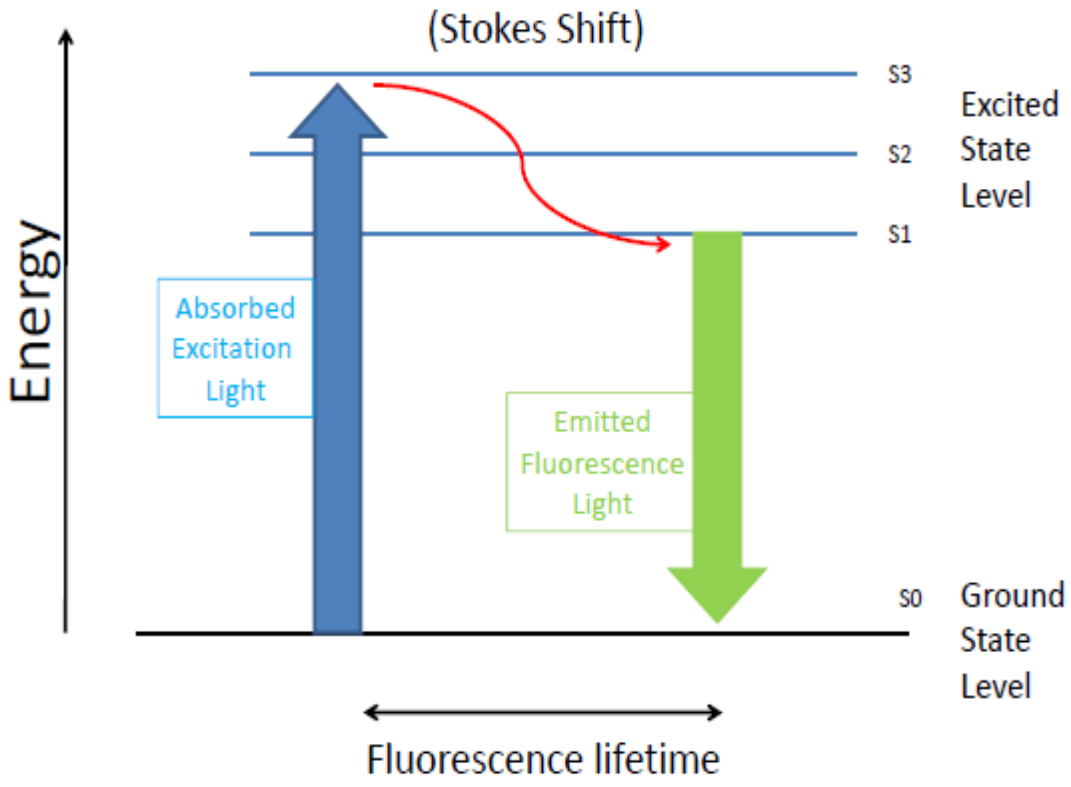

Figure 1.2: A detailed EEM map with associated common excitation-emission pairings that correlate to DOM sources and characteristics. The z-component is represented by the color bar to the right which quantifies the fluorescence intensity found amongst the different fluorophores found in the aquatic sample.

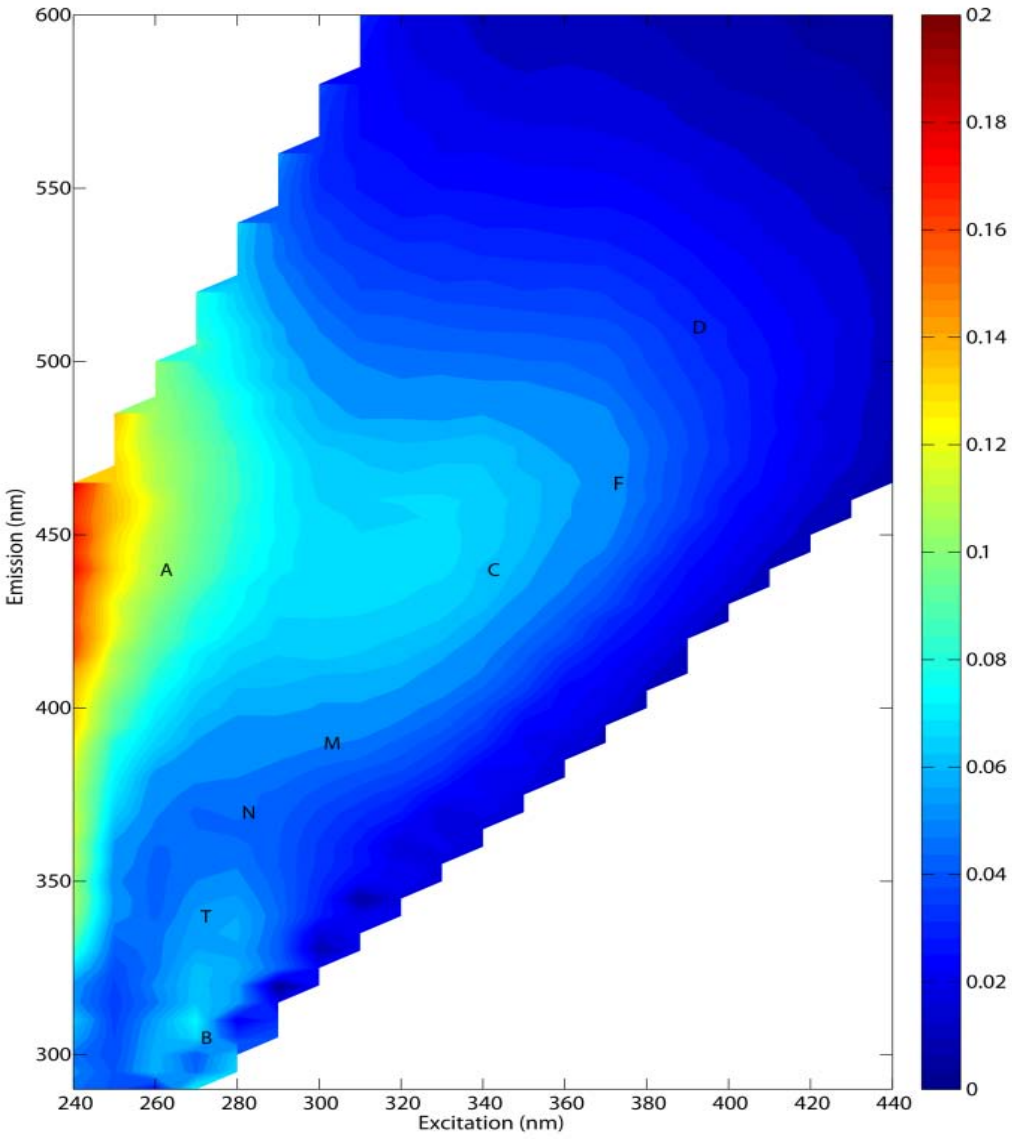

Figure 1.3: A set of protocols for EEM post-processing and the subsequent step procedures for implementation. The procedure starts with the collection of sample and blank reference and signal fluorescence data. First steps are to apply the manufacturer instrument correction files for the signal and reference detectors and then creating the corrected R1/S1 ratios. Next the Lacowitz correction (Lakowicz, 2006) is applied to the sample corrected data. After both the corrected blank and IFE-instrument corrected sample are normalized to the integrated area under the Raman signal. The final step is to subtract the corrected/normalized blank from the corrected/normalized sample.

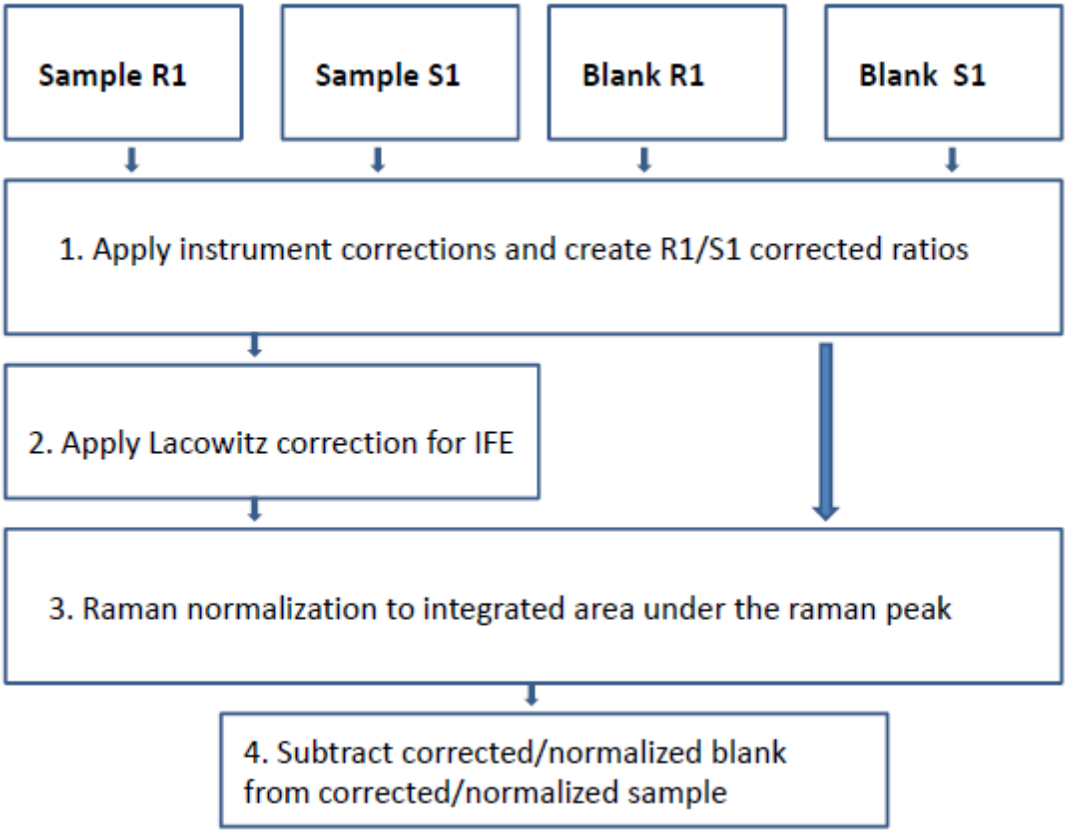

Table 1.1: Many key excitation-emission pairings and absorbance measurements have been identified that represent specific characteristics and sources of DOM and have been described throughout the DOM literature.

Fluorescence Peak/Parameter	**Excitation/Emission (nm)**	**Description**	**Reference**
B	270/306	Tyrosine like-protein like	Coble (1996,2007)
T	270/340	Tryptophan-like, protein like	Coble (2007), Stedmon et al. (2003)
A	260/450	Humic-like	Coble (2007), Stedmon et al. (2003)
M	300/390	Humic like, possibly marine	Coble (2007), Stedmon et al. (2003)
C	340/440	Humic-like	Coble (2007), Stedmon et al. (2003)
D	390/510	Soil fulvic acid	Stedmon et al. (2003)
Fluorescence Index (FI)	Ex370→Em470/Em520	Higher values indicate algal(microbial) vs. terrestrial derived DOC	McKnight et al. (2001)
$SUVA_{254}$	Absorbance at 254nm normalized to DOC	Correlated to aromatic content	Weishaar et al. (2003)
Spectral Slope $S_{275-295}$	Slope of absorbance over the range of 275-295nm	Lower $S_{275-295}$ correlated with higher aromatic content and higher molecular weight	Helms et al. (2008)

Chapter 2: Applications of Fluorescence Spectroscopy to Predict Wastewater in an Urban Stream

(Article appears as submitted to *Environmental Science and Technology*. Co-authors are Stewart Rounds and Joseph Needoba)

2.1 Abstract

In an urban watershed anthropogenic influences can cause detrimental effects on the region's rivers and streams and lead to negative impacts on water quality. Determining the water quality and health of these aquatic ecosystems requires identification of natural and anthropogenic influences and an understanding of the seasonal hydrologic cycle. Dissolved organic carbon (DOC) represents a significant carbon reservoir in all ecosystems and can be used as a means to measure the characteristics and sources of organic matter in aquatic environments. Fluorescence spectroscopy can be used to quantify and characterize a subset of the DOC pool, the colored dissolved organic matter (CDOM), which can absorb and re-emit energy as fluorescence. This study utilizes fluorescence spectroscopy to characterize organic carbon in the Portland, Oregon urban watershed temporally and spatially and to trace the anthropogenic signature found in wastewater effluent associated with treatment plants. Samples were collected from multiple sites within and outside the urban area and from effluent of two different wastewater treatment plants. Several statistical approaches were used to develop a robust model to predict the amount of wastewater present in a stream sample: end-member mixing experiments were conducted to demonstrate the linearity of fluorescence; principal component analysis was used to distinguish sources and characteristics of the organic matter; and a multivariate linear regression model was built using three key fluorescence peaks to characterize the organic matter. The model was validated and predicts the percentage of

wastewater in a sample within 80% confidence. The model results can be used to develop in-situ instrumentation, inform monitoring programs, and develop additional water quality indicators for aquatic systems.

2.2 Introduction

Naturally occurring and anthropogenic organic matters is the subject of intense scientific research and are critical to the understanding of many water quality problems. Determining the health of a river system requires identification of natural and anthropogenic influences on stream biogeochemistry and an understanding of seasonal hydrological variations. Organic matter (OM) is a critical component in many biogeochemical cycles including those of nutrients and trace metals. Organic matter is ubiquitous in aquatic environments and can significantly affect ecosystems in many ways. For example, OM can limit the availability of light in the water column, thereby directly affecting photosynthesis and phytoplankton growth, which are important for the aquatic food web (Wetzel, 1992). Transport of toxic metals into the water column attached to the OM can lead to diminished biological health and can increase sediment oxygen demand, thereby depleting the dissolved oxygen available for fish (Thacker et al., 2005). Dissolved organic matter (DOM) is leached out of soils and decaying plants and animals, produced by algae in the water column, and augmented by anthropogenic inputs (e.g. wastewater discharge and agricultural production). For aquatic systems, sources of DOM are commonly categorized as: (1) allochthonous— material entering water from terrestrial, wetland, and littoral zones, and (2) autochthonous— material derived from biota (e.g. algae, bacteria, and macrophytes) within the water column (Aiken and Cotsaris, 1995, Reckhow et al., 2008). DOM is vital to the ecosystem because it is highly reactive and is an integral part of

microbial food webs, biogeochemical reactions, and UV light attenuation. Dissolved organic carbon (DOC), a main component of the DOM pool, represents a significant global carbon reservoir needed in all ecosystems and can be used as a water quality indicator (Battin et al., 2009).

Fluorescence spectroscopy has been used to quantify and characterize a subset of the DOC pool - colored dissolved organic matter (CDOM) or sometimes called fluorescent dissolved organic matter (FDOM) - which can absorb certain wavelengths of light and re-emit a fraction of that energy as fluorescence (Coble, 1996). Fluorescence measurements can be used to detect, quantify and 'fingerprint' the various sources of DOM. Because the interaction of a water sample with light is determined both by DOM concentration and composition, the benefit of measuring the optical properties of DOM is that such measurements provide additional information about the character, quantity, and sources of the DOM pool (Downing et al., 2009, McKnight et al., 2001). Fluorescence spectroscopy provides a rich source of information about the chemical characteristics of the DOM pool (Coble, 2007, Hudson et al., 2007a, Stedmon and Bro, 2008). Differences in those characteristics then can be linked to changes in DOM reactivity and may be used to infer DOM sources (McKnight et al., 2001). Qualitative information also can be derived from the identification of peaks produced from the full excitation-emission matrix (EEM) of fluorescence spectra-peaks that have been linked to different DOM pools such as humic and fulvic acids, protein-like material and phytoplankton-derived material (Coble, 2007, Hudson et al., 2007a, Stedmon et al., 2003).

As human population growth continues and urban boundaries expand, the demand continues to increase for clean healthy aquatic systems for food and drinking water. Over 130,000 km of streams and rivers in the United States are impaired by urbanization (Paul and Meyer, 2008). Urbanization has increased the presence of many anthropogenic effects on streams, causing increases in constituents such as oxygen demand, conductivity, suspended solids, ammonium, hydrocarbons, and metals in urban streams (Paul and Meyer, 2008). Anthropogenic inputs into the urban waterways are having adverse effects on water quality, aquatic ecosystems, and habitat. Tracking the sources of anthropogenic inputs by examining the characteristics of organic matter found in wastewater effluent is a necessary step towards monitoring and examining related impacts on water quality in urban streams.

The aims of this study were to (1) develop a robust model using measurable characteristics of CDOM to identify wastewater as an anthropogenic tracer and quantitatively predictive for the amount of wastewater effluent in aquatic systems, (2) demonstrate the linearity of fluorescence responsive with an end-member mixing experiment, (3) use a multivariate linear regression approach to quantify the amount of wastewater found in a sample, and (4) distinguish among the sources and characteristics of stream organic matter using principal component analysis.

2.3 Experimental Section

2.3.1 Site Description

The study was conducted in and around the Tualatin River Basin on the west side of the Portland Oregon metropolitan area (Figure 1). The drainage basin has an area of approximately

712 square miles with many tributaries entering the river representing a wide range of land uses. The river drops about 1800 feet during the first 12 miles and then only 250 feet for the remaining 80 miles, resulting in a relatively slow moving river on the valley bottom. In some portions of the river, the slope is negligible and such pooled reaches are suitable for the growth of large amounts of algae. The Tualatin River headwaters originate in the Coast Range; however, most of the precipitation in the watershed is in the form of rainfall and the snowmelt contribution is minimal (Bonn, 2009). Peak rainfall and higher flow occurs November through April, and the drier low-flow season occurs May through October. The watershed can be characterized into upper, middle, and lower reaches which consist primarily of forested, agricultural, and urban areas, respectively(Bonn, 2010).

Approximately 500,000 people reside in 15% of the Tualatin River Basin, primarily in the urbanized lower reaches of the river. Clean Water Service is the primary wastewater and stormwater management utility for the urban areas of the Tualatin River Basin. Clean Water Services treats approximately 60 million gallons of wastewater per day through the operation of four wastewater treatment plants (WWTPs) that are among the most advanced in the Nation at removing phosphorus and other constituents from wastewater. Both wastewater facilities provide advanced tertiary treatment to remove excess nutrient loading, reduce biochemical oxygen demands, and suspended solid loadings. The treatment includes a final disinfection step to kill any remaining bacteria prior to discharging the effluent to the river. Discharge from the WWTPs is monitored and controlled by Clean Water Services to help protect water quality in the Tualatin River.

The basin has active streamflow, precipitation, solar, and water quality monitoring stations along the river and tributaries. A group of water-resource management agencies meets regularly to discuss the flow and water quality of the river system and produces annual flow reports that accurately document streamflow, both natural and augmented, and calculates percent wastewater in the river during low flow periods. During low flow periods, the Tualatin River near its mouth can contain up to 40% treated wastewater (Bonn, 2009).

This study was conducted primarily in the lower urban reach of the Tualatin River (Figure 2.1); with the exception of one site outside the urban growth boundary in an agricultural/forested tributary representing an end-member site with no wastewater characteristics. The study sites included two wastewater treatment facilities (Durham and Rock Creek Advanced Treatment Facilities), three tributaries (Fanno, Beaverton, and Rock Creeks), one headwater site (East Fork Dairy Creek), and one downstream river site (Tualatin River at Oswego Dam).

2.3.2 Sample Collection and Analysis

Water samples were collected every three to four weeks at each of the study sites over an entire year, thereby providing information spanning a range of hydrological and seasonal changes (Figure 2.2). Stream samples were collected with a Wildco Teflon Kemmerer 1.2 L sampler and transferred into clean glass BOD bottles. Wastewater samples were collected as grab samples from the plants at the effluent outflow sites. All samples were filtered (Whatman GF/F, combusted at 450°C for 4 hours) through a glass-fiber filtration unit. The filtrate was

collected into combusted glass amber bottles with Teflon lined caps and stored in the dark at 4°C until analyzed. All samples were analyzed within 5 days of collection.

Fluorescence excitation-emission matrix (EEM) measurements were conducted using a Horiba Jobin Yvon Flouromax-4 spectrofluorometer. To obtain fluorescence EEMs, excitation wavelengths were incrementally increased from 240 to 450 nm at step intervals of 10nm, and fluorescence was measured at emission wavelengths of 300 to 600 nm at step intervals of 2nm. The excitation and emission slits were set to a 5-nm bandpass. A flow-through water bath was used to maintain a constant temperature of 20°C. UV-visible absorbance measurements were conducted with a J&M TIDAS spectrophotometer. Dissolved organic carbon (DOC) measurements were made using the platinum catalyzed persulfate wet oxidation method on an O.I. Analytical Model 700 TOC Analyzer (Aiken, 1992) at a U.S. Geological Survey laboratory in Boulder, CO. Specific ultraviolet absorbance at 254 nm ($SUVA_{254)}$ was determined by dividing the UV absorbance measured at 254nm by the DOC concentration and are reported in units of liter per milligram of carbon (Weishaar et al., 2003).

Several post-acquisition steps were used to adjust the EEM data. First, the excitation and emission data were corrected for instrument-specific response. Second, the EEM response of Milli-Q water was subtracted from sample EEMs. Third, the UV-visible absorption spectra were used to correct the EEM data for inner filter effects (McKnight et al., 2001). And finally, the fluorescence intensities of the EEMs were normalized to the area under the Raman peak, thereby converting the arbitrary units (AU) into Raman units (RU). The corrected EEM's were imported into Matlab R2010A (Mathworks) for further analysis, including removing portions of

the EEMs at which there was interference from Raleigh scattering, converting the data into vectors, and selecting characteristic peak signals based on documented key excitation/emission pairings (McKnight et al., 2001, Coble, 1996), and plotting the data into contour and surface maps. The fluorescence index (FI), which is the ratio of emissions at 470 nm to 520 nm at an excitation of 370 nm, was calculated for all samples [8].

All samples were concurrently analyzed for ancillary parameters of chlorophyll *a* and dissolved nutrients (nitrate, nitrite, phosphate, ammonium, and silicate). Nutrients were analyzed using a 5-channel 2008 model Astoria-Pacific segmented continuous flow injection analyzer designed for spectrophotometric analysis of nutrients in freshwater. Chlorophyll *a* was analyzed using a Turner Trilogy fluorometer.

2.3.3 Modeling Approach

Wastewater and other anthropogenic sources such as fluorescent whitening agents (FWA) have been documented to influence specific regions of the EEMs at peak A (excitation at 260 nm and emission at 450 nm [ex260/em450nm]), peak T (ex270/em340nm) and peak C (ex340/em440nm) (Hudson et al., 2007b, Saadi et al., 2006). The EEMs from samples collected from the Durham Rock Creek and WWTPs in the Tualatin River Basin show these three peaks with the strongest fluorescent intensities (Figure 2.3); therefore, these three peaks were chosen for further analysis and modeling in this study.

2.3.3.1 End-Member Mixing Experiments- Laboratory mixing experiments with two end-members, one effluent sample and one headwater reference sample, were conducted to determine the fluorescence response of mixed samples and the degree of linearity in that

response. Samples were collected from both end members (East Fork Dairy Creek and both WWTPs) into combusted 1-L amber glass bottles and filtered according the procedures described above. Sample mixtures were prepared in 40mL combusted amber glass vials. Two experiments were conducted (one per WWTP), and 10 samples were prepared with 10 percent increments of wastewater per sample. Samples were mixed and shaken for 2 hours at room temperature prior to analyses. Fluorescence and absorbance measurements were taken and post-EEM acquisition steps were applied.

2.3.3.2 End-Member Mixing Model - Three approaches were used to analyze the results from the stream samples. In the first approach, the potential for individual peaks to predict percent wastewater was assessed utilizing an end-member mixing model. Rearrangement of a mass balance equation for a two end-member system was used to calculate percent wastewater at the downstream river site, Tualatin River at Oswego Dam (Schemel et al., 2006).

$$\frac{\qquad\qquad\qquad}{\qquad\qquad} \tag{1}$$

Where upstream represents the fluorescence signal from the headwater site as an indicator of upstream conditions not affected by wastewater. Downstream represents the fluorescence signal at the downstream mixed site, and WW_1 and WW_2 represent the fluorescence signals from the Rock Creek and Durham WWTP samples. In this analysis the fluorescence intensities for peaks A (ex260/em450), T (ex270/em340), and C (ex340/em440) were identified and used in the equation above. The calculated percent wastewater in the Tualatin River at Oswego Dam

from this model was compared to the actual percent of wastewater computed for that site from the flow measurements.

2.3.3.3 Multivariate Linear Regression Model- The second approach for analyzing stream sample fluorescence data to quantify percent wastewater was to use signals from peaks A, T, and C together in a multivariate linear regression model. The statistical analysis package Minitab16.1.1 (Minitab Inc.) was used to develop this model. Multivariate linear regression is a method that is used to model a relation between two or more explanatory variables and a response variable through a linear combination of the explanatory data. In determining the best possible multivariate regression model, an exploratory analysis was conducted using all of the key peaks (A, C, M, B, and T) identified in the OM literature (Coble, 1996, Fellman et al., 2010). That analysis indicated that the strongest model could be constructed based on peaks A, T, and C. Other model scenarios then were conducted to find the most robust model, such as using peak signal ratios and differences to minimize multicollinearity issues, alternating the assumptions made on tributary sample wastewater percentages, and varying the model input sample size. Based on an analysis of model goodness-of-fit statistics and use of the variance inflation factor to quantify multicollinearity issues, the strongest model was produced using an unmodified signal from peak A, and the peak signal ratios A/T and A/C as explanatory variables, and an assumption that the tributary samples contained no wastewater. Model inputs incorporated data from 74 samples; including 12 headwater samples (East Fork Dairy Creek), 28 tributary samples (Beaverton, Fanno, and Rock Creeks), 11 downstream river samples (Tualatin River at Oswego Dam), and 23 wastewater effluent samples (Rock Creek and Durham WWTPs). Upriver samples and tributary samples were assumed to contain zero percent wastewater for

the model. The percent wastewater in downstream river samples was calculated using methods applied by Bonn in an analysis of Tualatin River flows(Bonn, 2009). The model was tested with 30 separate samples including 17 samples collected from a site on the Clackamas River found outside the urban growth boundary (zero percent wastewater) and 13 samples collected from the Tryon Creek WWTP (100 percent wastewater).

2.3.3.4 Principal Component Analysis- The third approach for analyzing the fluorescence data was to use principal component analysis (PCA) to explain some of the strongest patterns in the data used to create the multivariate linear regression model. PCA was conducted using the Minitab 16.1.1 software (Minitab Inc.) and using 3 variables (peak intensities for A, T, and C) for the same 74 samples used in the regression model. To better analyze the patterns in the data, the peak intensities were normalized prior to PCA analysis by subtracting the mean of the peak intensities (separately for Peaks A, T, and C) and dividing by the standard deviation. PCA scores were computed and are graphically represented by plotting the first principal component against the second.

2.4 Results

2.4.1 End Member Mixing Experiments- A linear response in fluorescence peak intensity was confirmed for Peaks A, T, and C from the results of the end-member mixing experiments (Figure 2.4). As the percentage of wastewater in each mixed sample increases the peak intensities increase linearly. The strength of the linear response varies for each peak and slightly varies from one wastewater treatment plant to the other. Based on this confirmation of a linear fluorescence response, an end-member mixing model would work well for the results

of the end-member mixing experiments, and may or may not be successful in predicting results for the downstream river site, depending on the extent to which OM sources can be categorized into two major groups (headwater and wastewater). If, however, the downstream river samples have OM sources and characteristics that span multiple important sources, then the two component end-member mixing model may break down regardless of the linearity of the fluorescence response.

2.4.2 End-Member Mixing Model – The two-component end-member mixing model was applied to predict the percent wastewater at the downstream river site, but the results were not optimal (Figure 2.5). An optimal model would predict values that fall on the black solid 1:1 line, or as close to that line as possible to demonstrate accuracy for predicting the presence and fraction of wastewater. Results from three separate end-member mixing models, using fluorescence intensities from peaks A, T, and C, show that predicted wastewater percentages indeed tend to increase as actual wastewater percentages increase, but the model predictions are not particularly accurate with this simple approach. For comparison, results from the multivariate linear regression model for the same samples are also plotted on figure 5. Analysis of the model performance shows that the multivariate regression model provides a better overall prediction of the percentage wastewater for most samples. It is likely that the two-component end-member mixing model fails to predict the percent wastewater accurately because sources of OM in the basin are many and varied, and are not fully characterized by an assumption that most of the fluorescence signal is captured by just wastewater and headwater sources.

2.4.3 Multivariate Linear Regression Model- Results from the multivariate linear regression model demonstrate that fluorescence signals can be used to predict the percentage of wastewater at a downstream site with sufficient accuracy to be useful. Summary statistics for the model predictions are provided in Table 1. The full multiple linear regression equation for the model is:

(2)

Where %WW is the predicted percentage of wastewater in the sample, and Peak A, C, or T is the fluorescence intensity at the indicated peak in the excitation-emission matrix. Plotting the predicted versus the actual wastewater percentage for all the samples used in the model shows that the model performs relatively well (Figure 2.6). A linear regression on those results indicates that the model captures about 95% of the variability in the data (R^2=0.9503).

The dashed blue line in Figure 2.6 shows that 95% of the samples can be predicted within 80% accuracy. The overall mean error and mean absolute error of the model are 0.10% and 8.1%% respectively. For an optimal model, the mean error should be zero to indicate a minimum of model bias, and the typical error as expressed by the mean absolute error and should be small enough (≤ 10%) that the model predictions remain useful. The categorical statistics found in Table 2.1 show the model had the greatest predictive capabilities for the headwater and tributary sites with a mean error and mean absolute error of 4.7,7.0 percent and 1.2, 7.6 percent respectively. The downstream and WWTP samples show a greater deviation in the model with mean errors and mean absolute errors of 5.2, 9.2 percent and 3.2,

8.6 percent respectively. The model was tested with fluorescence data from a pristine Clackamas River site within 95% accuracy but over-predicted the percent wastewater character of Tryon Creek WWTP effluent (~ 125%) for all of the samples. Tryon Creek WWTP uses less advance treatment process compared to the Durham and Rock Creek WWTPs and has 40% more DOC in its effluent.

A comparison of actual and predicted percent wastewater at the downstream river site, Tualatin River at Oswego Dam, provides an opportunity to determine when and why the model deviates from the values calculated from the WWTP discharge measurements (Figure 2.7). The greatest divergence occurs at two time periods illustrated by the dashed red lines on figure 7- the first on June 29, 2009 and the second between the November 17 and December 16, 2009 samples. The bottom graph in Figure 2.7 illustrates some of the variations in nutrients and algal growth occurring during the time period of interest. The first point of the model's greatest divergence in late June under predicts the amount of wastewater and coincides with the peak of a significant phytoplankton bloom corroborated by chlorophyll *a* measurements of 20µg/L. As the bloom crashes both ammonium and phosphate recover to their highest levels found during the sampling period. The second period of greatest divergence (November and December of 2009), when the model is over predicting the amount of wastewater, corresponds to the first high flow events to occur after the summer low flow period (Figure 2.2). During this time period there is a notable increase in the amount of ammonium and phosphate in the system (Figure 2.7).

2.4.4 Principal Component Analysis- Principal component analysis was used to qualitatively assess the variability in the fluorescence data from the samples used in the multivariate linear regression model. The fluorescence data from peaks A, T, and C were first normalized prior to running the PCA analysis, and although the normalization might remove some of the patterns in the ratios of the fluorescence data, the resulting PCA analysis provides some useful information to help understand how the OM characteristics vary among sites and samples. A plot of principal component 2 (PC2) against principal component 1 (PC1) shows that the downstream river, headwater, tributary, and wastewater samples all tend to cluster together (Figure 2.8). PC1 captures 83% of the variability in the fluorescence data, and 16% of the remaining variability can be explained by PC2, which accounts for 99% of the variability found in the dataset.

Some of the clustering and trends in the plot of PC2 versus PC1 can be explained by examining the characteristics of the OM in these samples. The characteristics of the OM as measured by DOC concentration, $SUVA_{254}$, and FI vary according to the sample site category and the time of year or flow conditions (Figure 2.9). The WWTP effluent samples typically provided the highest DOC concentration, highest FI value, and the lowest $SUVA_{254}$. Seasonally, the WWTP effluent has the greatest amount of DOC during the high-flow time period, which is consistent with the fact that the most extensive wastewater treatment at these facilities occurs during the low-flow summer conditions to protect the river's water quality during those critical conditions. The tributary samples have the second highest DOC concentrations, the lowest FI values, and the highest $SUVA_{254}$. Contrary to the WWTP results, the tributary samples have the highest concentrations of DOC during the low-flow season, possibly due to the algal growth and

heightened level of bacterially mediated OM processing during the warm weather months. The headwater samples have the lowest concentration of DOC, but values of $SUVA_{254}$ and FI that are similar to those from tributary samples. Seasonally the headwater site has the highest concentrations of DOC during the high flow period, which might reflect the mobilization of OM from the streambed or transported to the stream from nearby soils or riparian areas. Finally the downstream river samples exhibit mid ranges for all the parameters which demonstrate that the mixture is truly a combination of the WWTP and headwater/tributary samples with a mixture of sources and OM characteristics. Seasonally, the highest DOC concentrations and the highest SUVA254 values from the downstream river site were during high flow conditions.

2.5 Discussion

The main assumption of the two-component end-member mixing model is that if a sample is simply a mixture of two components, then the downstream river mixed site should produce a simple linear fluorescence response of the two upstream components, in this case a headwater and a WWTP effluent signal. The results of the two-component end-member mixing model, however, failed to match the actual percent wastewater data. The individual models based on peaks A, T, and C does trend in the correct positive fashion but all three mixing models overestimate the percent wastewater for every sample. Peak T has been considered a tracer of anthropogenic material in previous studies and has been identified as being highly correlated to the biodegradable fraction of wastewater (Reynolds and Ahmad, 1997). In the end-member mixing model, Peak T had the strongest predictive capabilities compared to the models based on Peaks C and A. The primary problem, however, is that the downstream river sample is not simply a mixture of the headwater and WWTP waters but is a complex

heterogonous mixture of many different sources and characteristics of organic matter that requires a more complex modeling approach.

Application of a multivariate linear regression model to the fluorescence data has several advantages over the two-component end-member model. For example once a model has been constructed and tested for a system, only one discrete sample is required to make a prediction with the model. Such a prediction to determine percent wastewater in the Tualatin River Basin can provide near 80% accuracy and perhaps even greater accuracy during low flow (higher wastewater) time periods. In addition, the multiple linear regression approach provides the ability to quantify more complex mixtures of organic matter entering a system using multiple excitation/emission pairings. Using multiple peaks (A, T, and C) allows the model to capture signatures from more humic- terrestrial derived organic matter, anthropogenic, and microbially derived components (Baker and Spencer, 2004). Constructing a model across a range of locations and seasons also allows the model to capture a changing menu of various sources of organic matter across a range of hydrological conditions.

Closer examination of the categorical statistics from the multiple linear regression model shows the greatest errors occur for samples from the downstream river site and the WTTPs. One model assumption was that certain OM signatures were sufficient to categorize an effluent sample as 100% wastewater; however, that assumption does not take into account the complexity and variability that occurs seasonally due to changing source materials and treatment processes and variations from one WWTP to another. Both treatment plants included in this model use advanced tertiary processes during summer and are exposed to

similar meteorological influences, but they service different clientele in the urban area and have slightly different treatments that could cause variations in the effluent fluorescence signatures. At the time of the sample collection, the Durham WWTP utilized biological methods of nutrient removal of phosphorus and ammonia, along with a reduced application of alum and lime (to further remove phosphorus), while the Rock Creek WWTP did not employ the same biological removal processes and therefore was using twice as much lime and alum. Seasonally, both WWTPs have higher inflows during wet seasons because of stormwater and groundwater influxes into the collection system, resulting in seasonally varying contributions of anthropogenic sources and terrestrial humic sources to the WWTPs. During the winter high-flow periods both WWTPs typically reduce their treatment processes to secondary levels because the critical treatment period to protect riverine water quality is the summer low-flow period. During the sampling year, the WWTPs converted to secondary levels on November 1st, 2009, which coincides with the second time period of greatest divergence found in the model. The increase in ammonium and phosphate during this time supports an increase in the effluent OM influence on the downstream river samples. The model over predicts wastewater during this time period; however, the change in treatment processes was not accounted for in the model through the use of a time-varying term or other seasonal categorical classification in the model.

The heterogeneous mixture of compounds found in wastewater effluent coming from domestic and industrial waste, and periodically from stormwater and groundwater sources has a distinct fluorescence signature that can still be identified in a more complex mixture of aquatic organic matter. Ammonium and phosphate have been described as nutrients found in

increased abundance in urban streams due to the presence of wastewater effluent influence (Paul and Meyer, 2008, Smart et al., 1985). Figure 2.7 shows that the model underpredicts the amount of wastewater during the peak of a phytoplankton bloom which greatly alters the source distribution of OM in the river at that time which is very different from the fluorescence characteristics of OM in the effluent.

The principal component analysis demonstrates the high information content inherent to peaks A, T, and C in the excitation-emission matrix to delineate the different sources and characteristics of the varying organic matter in this sample set. The ability to use a model for predictive capabilities requires that distinct features be traceable in aquatic samples. The heterogeneous mixture of compounds found in wastewater effluent has a distinct fluorescence signature and distinct characteristics that are different from those in river samples. The effluent mixture exhibits high DOC concentrations with a low $SUVA_{254}$ value, indicating more labile and less aromatic carbon structures. The fluorescence index (FI), calculated as the ratio of emissions at 470 nm to 520 nm at an excitation of 370 nm, has been widely used to indicate the relative contributions of algal versus terrestrially derived DOM; higher FI are associated with algal derived material which has lower aromatic content and lower molecular weight, while lower FI values are associated with more highly processed, terrestrial derived material that has greater aromatic content and higher molecular weight (McKnight et al., 2001, Jaffé et al., 2008). The WWTP samples all contained a FI value greater than 1.66, identifying the OM in those samples as having a more microbially derived composition. The tributary samples also carry a distinct fluorescence signature characteristic of dissolved humic substances that consist of compounds such as lignin, tannins, and polyphenols which more commonly compose the bulk of humic

DOM fluorescence (Del Vecchio and Blough, 2004, Fellman et al., 2010, Green and Blough, 1994). The tributary samples also consisted of higher DOC concentrations similar to that of the WWTP samples but distinctly different with a low FI value and a high $SUVA_{254}$ indicative of less labile carbon and more aromatic structures. The upriver samples exhibited the lowest DOC concentrations and $SUVA_{254}$ and FI values similar to that of the tributary samples. The similarity between the tributary and headwater organic matter characteristics is consistent with OM sources that are more natural and less anthropogenic. The downstream river samples showed characteristics in the mid-range for DOC concentrations, $SUVA_{254}$, and FI values supporting the idea that the organic matter found at this site is really a complex mixture with sources that might have been sufficiently characterized by the headwater, tributary, and WWTP samples.

2.6 Environmental Implications

The use of a model to predict the presence and quantity of wastewater in a stream sample has many significant environmental implications for water quality monitoring. Recent advancements in sensor technology and the development of reliable and specific fluorescence probes have increased our ability to monitor OM characteristics in great detail and in near real-time. Custom sensors have already been constructed to capture the key peaks used in this study. The application of real-time data and constructed models such as the one presented in this paper will allow water managers to monitor and track point and non-point sources of pollution to aquatic systems. Even with varying accuracy this model enables detection of unknown anthropogenic inputs and may be useful in helping to identify problem areas or issues that might otherwise go undetected. Many urban areas have increasing concerns with organic wastewater contaminants (OWC) such as hormonally active chemicals, personal care products,

and pharmaceuticals that are designed to stimulate a physiological response in humans, plants, and animals (Kolpin et al., 2002). However, analytical analysis of these OWC's are often cost prohibitive and can only be done on a limited basis. The ability to utilize a fluorescence model to predict the presence and quantity of wastewater in a stream and determine periods of elevated anthropogenic inputs can be used to determine the most opportune times to investigate the suite of more expensive analyses such as OWC's and other anthropogenic pollutants in aquatic ecosystems.

Chapter 2 Figures

Figure 2.1: Map showing the sites sampled in this study. Most stream sites were located in or near the urban growth boundary Portland, Oregon. Stream sites generally were co-located with USGS water quality monitors.

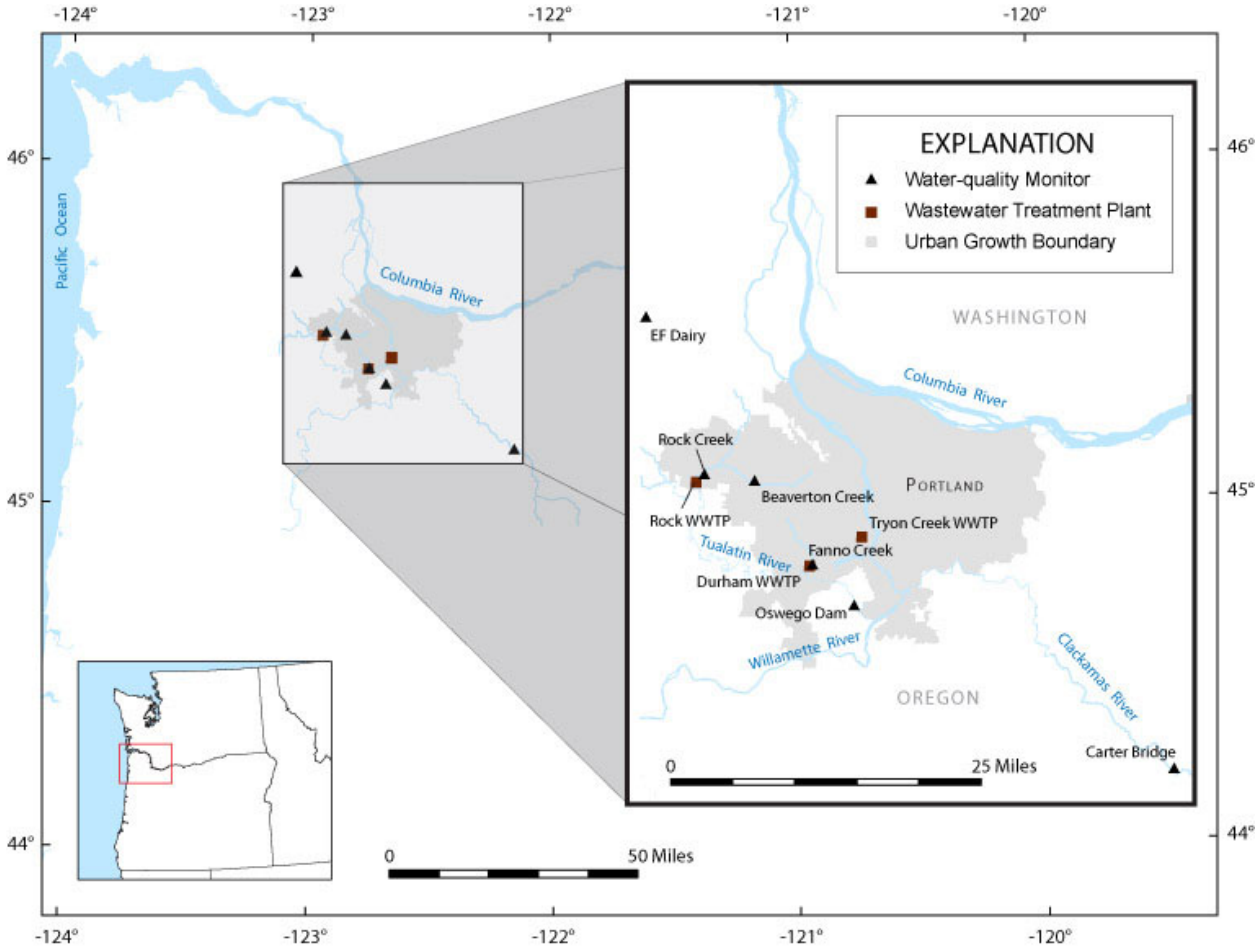

Figure 2.2: Graph of streamflow in the Tualatin River at West Linn (near Oswego Dam sampling site), showing the typical annual variation in streamflow, with higher flows during the November-April rainy season and low flows during the May-October summer period. The red dots represent the sampling dates.

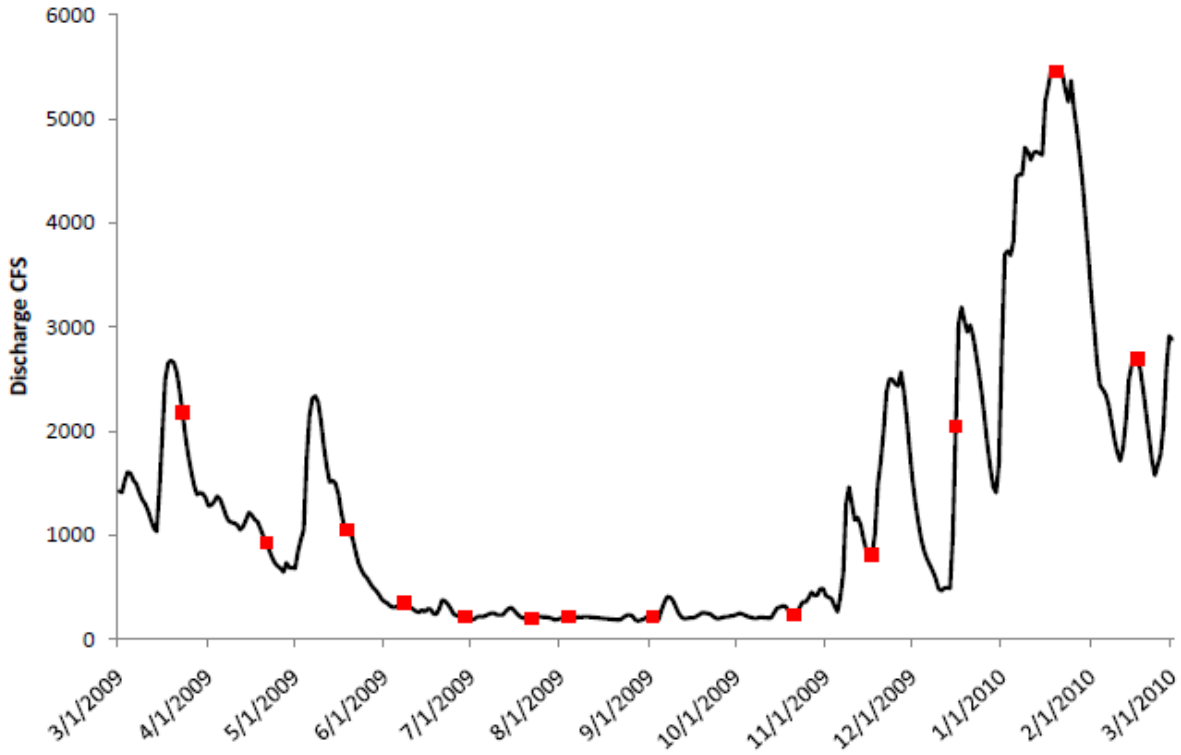

Figure 2.3: Sample excitation-emission matrices (EEMs) from June 6, 2009 from the headwater site (East Fork Dairy Creek), one of the wastewater effluent sites (Rock Creek WWTP), and downstream site (Tualatin River at Oswego Dam). The color scales for all three EEMs are identical and set to a range of 0-1.0 intensity.

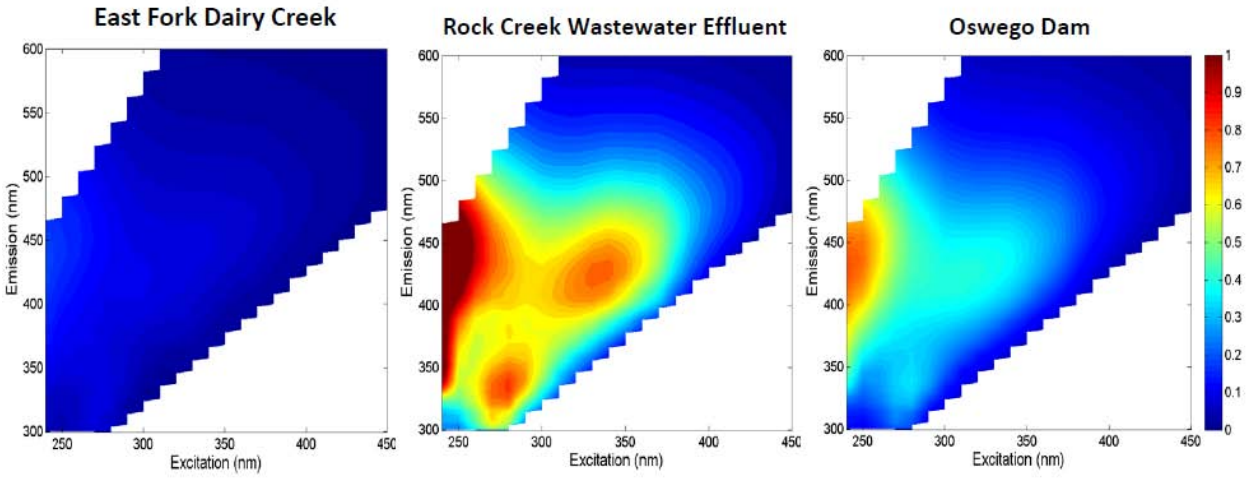

Figure 2.4: Results from end-member mixing experiments using water from East Fork Dairy Creek and water from a) Durham Wastewater Treatment Plant effluent and b) Rock Creek Wastewater Treatment Plant effluent. Signal intensities from peaks A, T, and show that the fluorescence response is linear as the percentage of wastewater in the mixed water sample increases.

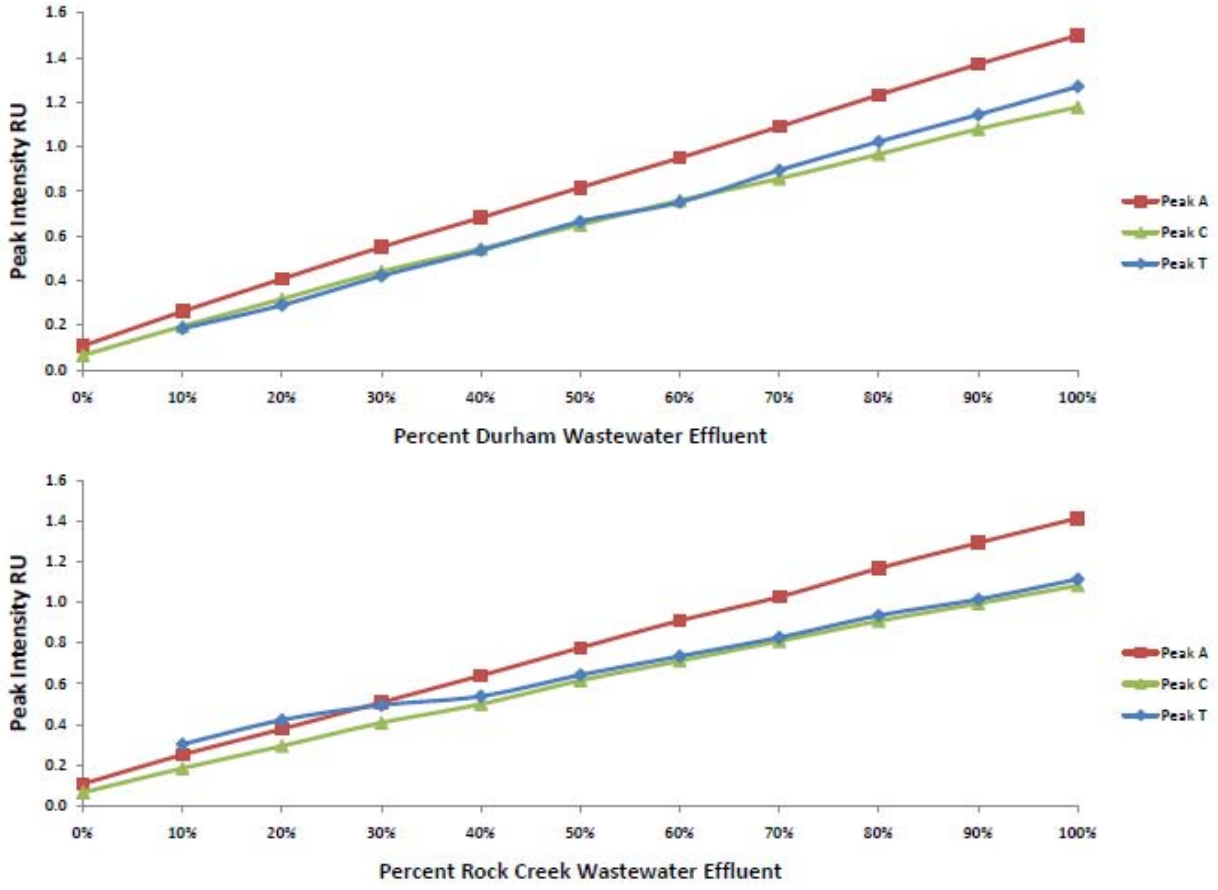

Figure 2.5: Predicted wastewater model results plotted versus actual wastewater found at Oswego Dam. The black line represents an ideal linear 1:1 line response. The blue diamonds are results based on the multivariate regression model. The individual peaks are the results from the two component wastewater mixing model.

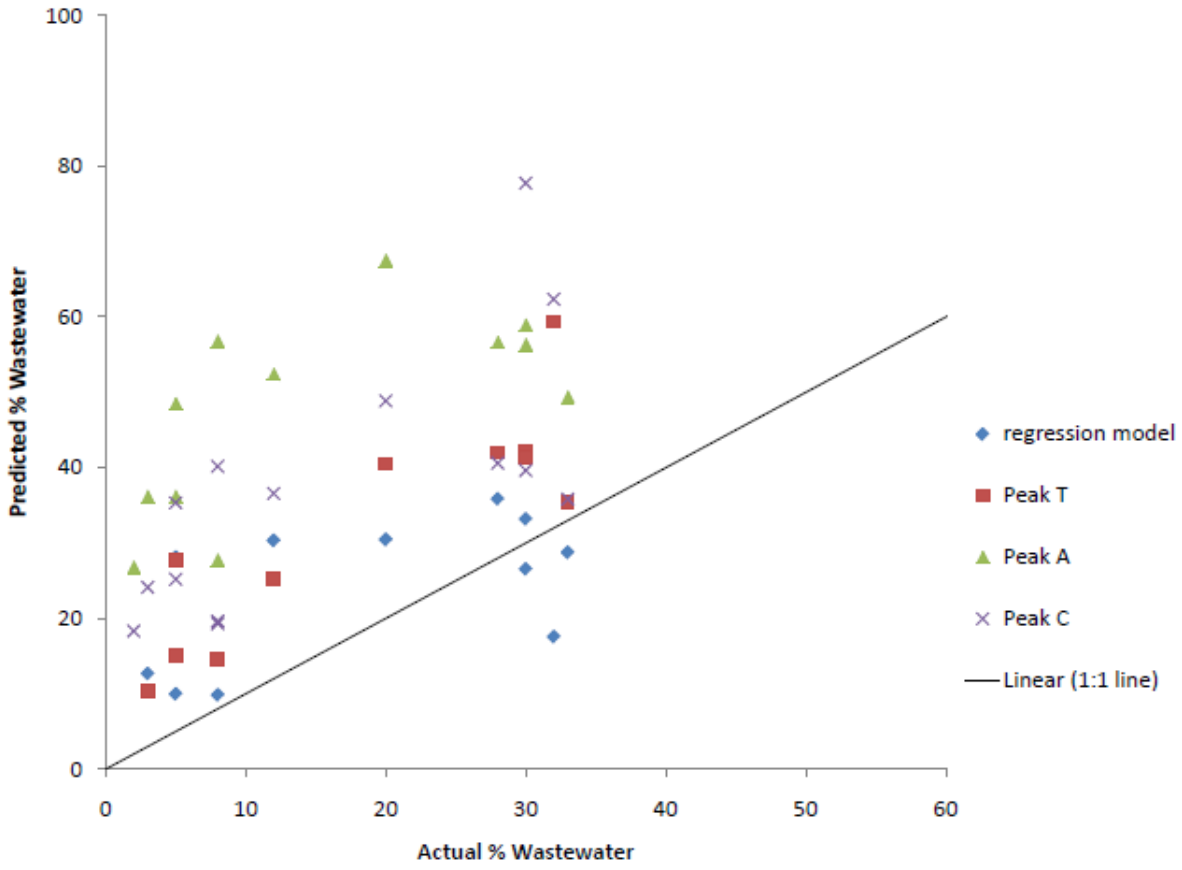

Figure 2.6: Results from the multivariate linear regression model for percentage wastewater over a range of sample conditions, where effluent samples are plotted at 100% wastewater, tributary and headwater samples are plotted at 0% wastewater, and river samples downstream constitute the points in between. The black line is a linear regression line through the results and the light blue boundary lines represent the 80% prediction interval.

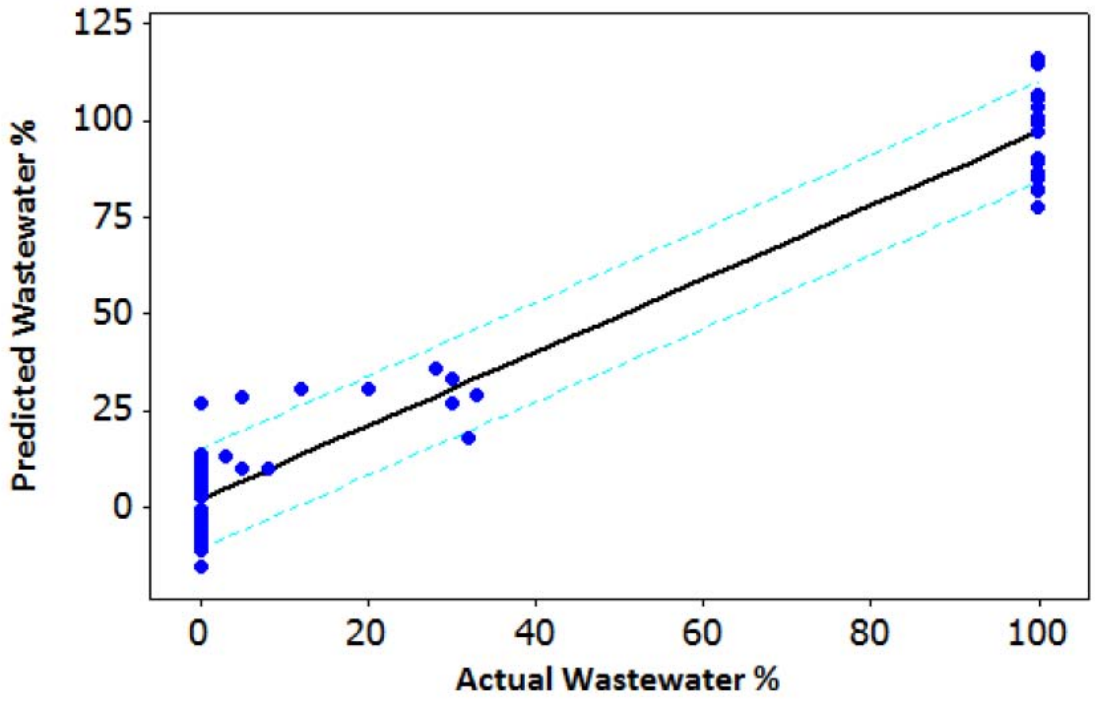

Figure 2.7: Results from the multivariate linear regression model for the downstream river site, Tualatin River at Oswego Dam. The top graph shows the modeled predicted percent wastewater results with the actual percent wastewater. The bottom graph shows phosphate, ammonium, and chlorophyll *a*. The red lines indicate the greatest divergence between the predicted and modeled results.

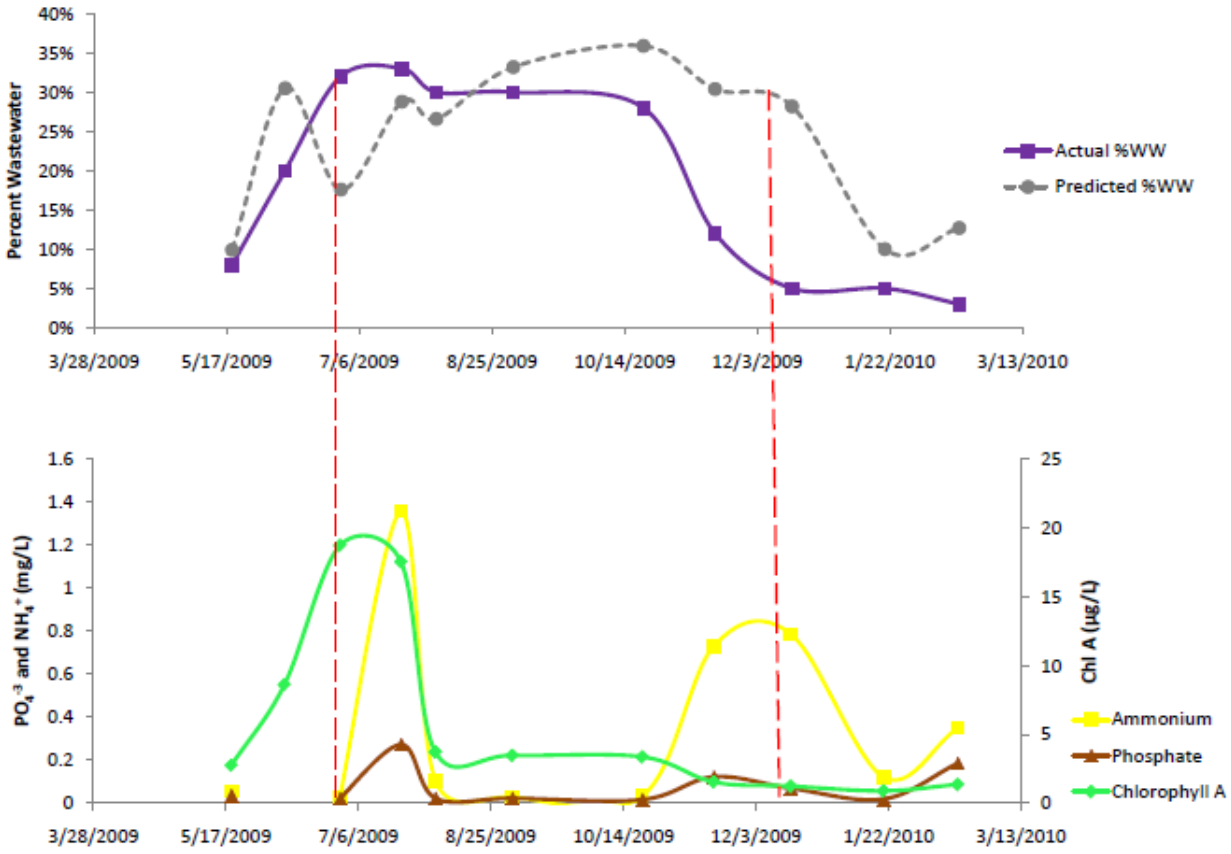

Figure 2.8: Plot of principal component 2 (PC2) versus principal component 1 (PC1) from a principal component analysis of the fluorescence data from Peaks A, T, and C. Sites shaded in the orange are effluent samples from wastewater treatment plants, light blue shaded data represents headwater site, shaded dark blue data represents the downstream river site, and the yellow shaded data represent the tributary sites. The blue outlined areas all have a fluorescence index (FI) value <1.66 and the ref values >1.66. DOC concentrations increase with increasing PC1, while $SUVA_{254}$ values decrease as PC2 increases.

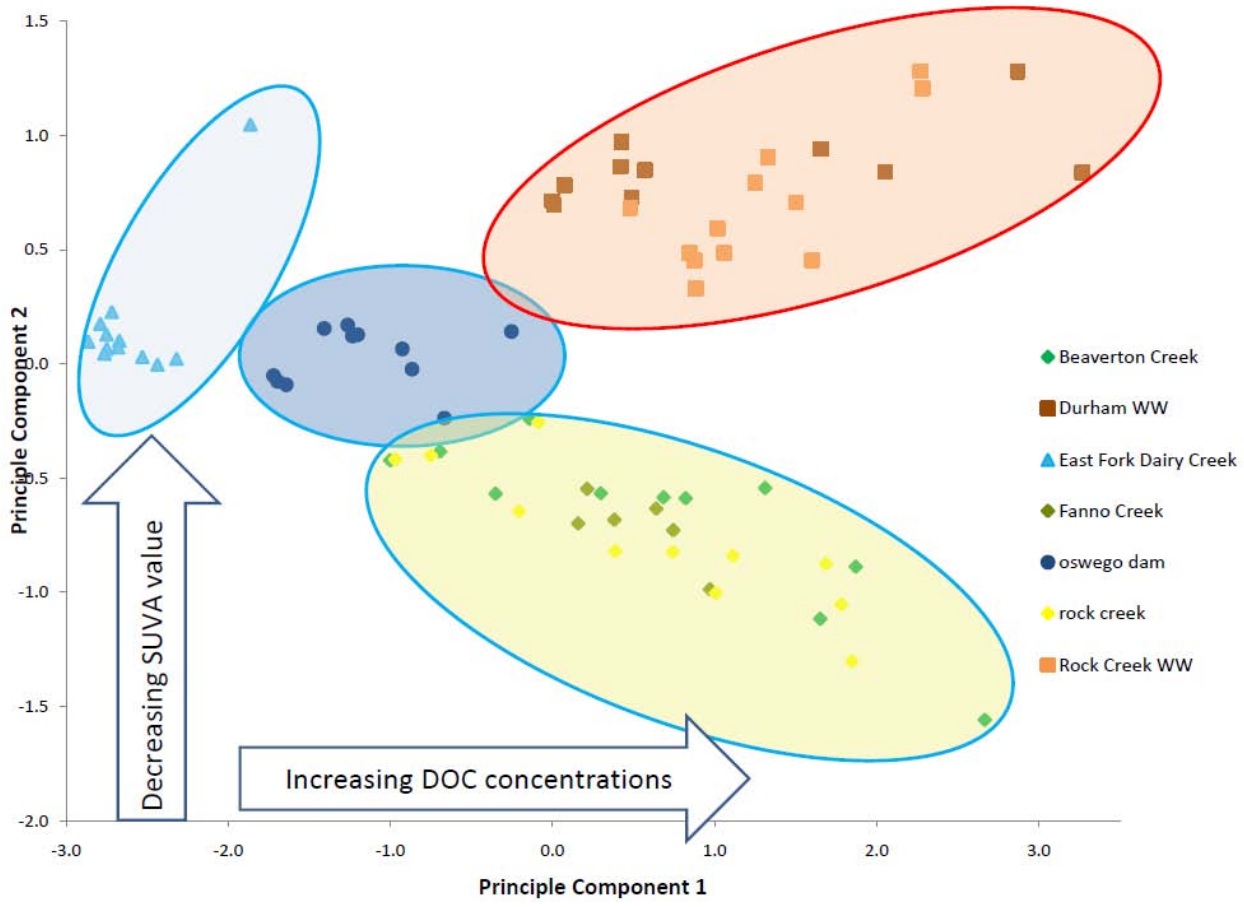

Figure 2.9: Summary graphs for averages of DOC, $SUVA_{254}$, and FI values. Top left graph and bottom graphs show averages for high, medium, and low-flow conditions. The graph on the top right shows year-long averages for all three parameters with DOC and $SUVA_{254}$ on the left Y axis and FI value on the right Y axis.

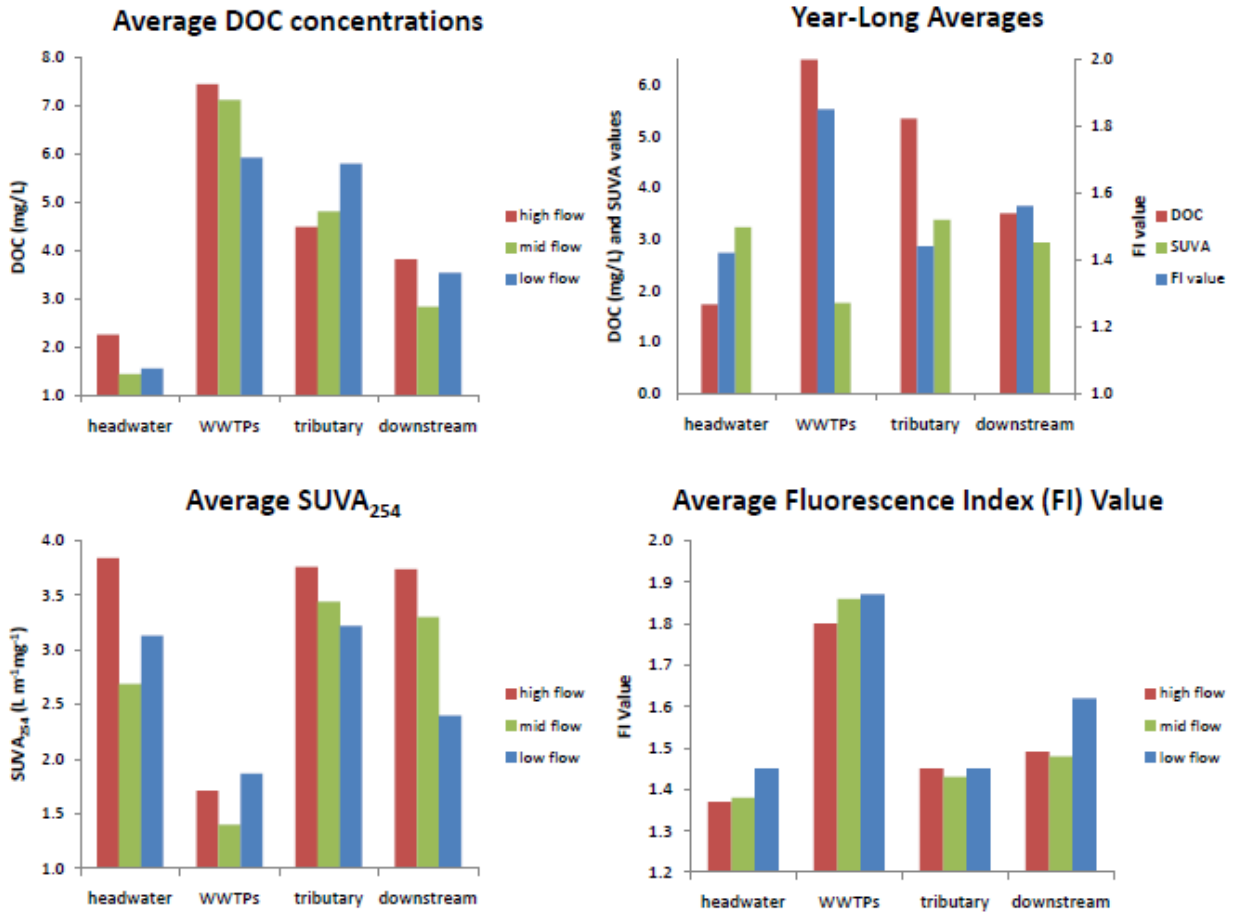

Table 2.1: Resulting statistics for the multivariate linear regression model using fluorescence signals at peaks A, T, and C.

Multivariate Linear Regression Model Statistics				
Variance Inflation Factor (VIF) = 1.22, 3.22, 3.41 Sample Size = 74 Mean Error = 0.1% Mean Absolute Error = 8.1% Samples with >15% error = 11 of 74 Samples with >20% error = 3 of 74				
Statistics	**Headwater Site**	**Downstream River Site**	**Tributaries**	**Wastewater Treatment Plants**
Sample Size	12	11	28	23
Mean Error	4.7%	5.2%	1.2%	3.2%
Mean Absolute Error	7.0%	9.2%	7.6%	8.6%
Samples > 15% error	0	2	2	7
Samples >20% error	0	1	1	1

Chapter 3: Future Research

Two current research initiatives are ongoing as an extension to this thesis research. Both focus on the Portland, Oregon watershed and involve the implementation fluorescence spectroscopy as a method of identifying sources and characteristics of organic matter and potential impacts on urban water quality issues.

The first project aims to identify and characterize sources of organic matter that may impact drinking water in Clackamas River, Oregon. The objectives of this study are to, (1) characterize the quantity and quality of organic carbon in the Clackamas River, its major tributaries, and in North Fork Reservoir, (2) establish the relationship between dissolved organic carbon (DOC) and DBP precursor concentrations at sites throughout the watershed (and in finished drinking water), (3) identify potential sources of DBP precursors in the Clackamas River Basin, including, for example, fir and alder forest soils, algae, septic tank and waste-water treatment plant (WWTP) effluents, (4) assess the use of optical proxies, including data from in-situ fluorescent dissolved organic matter (FDOM) sensors, for the determination of DOC, THM and HAA precursor concentrations, and (5) examine treatability and removal of DOC and DBP precursors during “Jar Test” experiments. This study is a US Geological Survey lead project in collaboration with Clackamas River Water and Lake Oswego Drinking Water Plants. The research is led by Kurt Carpenter, Tamara Kraus, and myself and is ongoing with only preliminary results currently available.

The second project is to identify and quantify the sources of organic matter and fine organic-rich sediment to Tualatin River tributaries and quantify the amount of organic matter

residing in and moving through the streams. Samples from potential organic matter sources, such as leaf litter, soil, and stream bank materials, are being collected and analyzed for carbon content using stable isotopes of carbon and nitrogen, and fluorescence spectroscopy. This study explores the potential uses of fluorescence techniques to differentiate among organic matter sources and begin to correlate in-stream organic carbon measurements with fluorescence response. Detailed fluorescence analyses of samples are being collected over a wide range of hydrologic conditions, such as low-flow and storm events; to create and refine fluorescence correlation models used with in-stream real-time flourescence monitors. The in-situ monitors will assist in calculating carbon fluxes in and out of the system. This study is led by US Geological Survey in collaboration with Clean Water Services. The work is being conducted by Stewart Rounds, Jim O'Conner, Steve Sobieszczyk, and myself and is currently ongoing and results are not available at this time.

Fluorescence spectroscopy has advanced our study and understanding of organic matter sources, characteristics, transformations and transportation through aquatic ecosystems however, this method has some limitations. The detailed specificity of molecular components of NOM that can be achieved with high resolution analysis methods cannot be achieved with fluorescence alone. Current biogeochemical research is heading towards finding a way to interconnect and link the high resolution techniques with fluorescence spectroscopy technology. For example, a recent study tracing DOM as it moves from terrestrial porewaters into a Brazilian estuary uses high field Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR MS) for molecular characterization of the DOM and tracing the DOM characteristics and changes using fluorescence spectroscopy (Tremblay et al., 2007). The

combination of the two technologies allows for high resolution information about the molecular components and can trace the dynamics of the DOM pools as they move through the system.

A powerful tool to continue the advancement of fluorescence spectroscopy is to develop model correlations and statistical relationships with other more expensive discrete analyses. There are many research explorations of this nature currently underway. For example lignin phenol analysis has been proven to be a powerful biomarker and tracer of terrestrial DOM but an extremely complex analysis that limits spatial and temporal studies. When lignin analysis is coupled with fluorescence spectroscopy the ability to predict lignin concentrations and compositions can greatly extend research capabilities in terrestrial freshwaters as well as coastal zones with large riverine inputs (Hernes et al., 2009, Spencer et al., 2007, Spencer et al., 2009). Ziegmann et al, used toxin lab discrete analysis of *Microcystis areuginosa* (MC-LR), a toxin associated with algal blooms, to form correlation models with fluorescence spectroscopy to provide a method to use for online monitoring of algal and cyanobacterial growth in water reservoirs used for drinking water (Ziegmann et al., 2010). Since fluorescence spectroscopy has been successful at distinguishing the microbially derived components of NOM another potential model correlation with E. *coli* bacteria can be explored. If a correlation exists, advance monitoring for lakes, rivers, and reservoirs can help detect and protect aquatic habitats and human health. Although many correlations and models using fluorescence spectroscopy and ancillary parameters exist, there are still many relationships and investigations to explore.

Advancement in fluorescence spectroscopy instrumentation and in-situ sensors has continued to progress the NOM research. Horiba Scientific has developed a new fluorescence spectroscopy instrument called the Aqualog, just released in March 2011, which is the only simultaneous absorbance and fluorescence system for water quality analysis. The Aqualog provides cutting edge analyses, 100-times faster sample processing, batch post-processing capabilities, and increased time efficiency and was developed specifically for the needs of the NOM fluorescence research scientists. Increased efficiency will allow fluorescence research to continue to grow and develop. In-situ sensor manufacturers, Wetstar and Turner, have been keeping up with the pulse of the research developments and continue to grow and expand with the research needs. For a current study in the Clackamas River, the scientists worked with Turner Design to develop new in-situ fluorescence probes based on key excitation-emission pairs that can characterize NOM beyond just the common FDOM probe. Studies like these help to trouble-shoot and problem solve the limitations that currently exist and provide necessary feedback to the engineers to continue to advance the development of these new sensors. Currently an instrument that can measure the full EEM spectra and not just single excitation-emission pairings is being developed which will take fluorescence monitoring to the next level.

If we take a historical look of the organic matter research significant accomplishments have occurred in the past 50 years. What was once an elusive mass of 'gunk' that went primarily uninvestigated can now be identified down to its molecular composition and can be traced and monitored spatially and temporally across aquatic systems. Defined roles and functions of NOM have been identified and investigated across ecosystems. In the next 50 years organic matter research will grow by leaps and bounds as technology advancements continue

to develop, collaboration efforts and research initiatives grow in the NOM community, and the role of carbon and its importance on a global scale remain in the forefront of science expeditions.

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