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Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review

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Abstract

The biochemical composition of dissolved organic matter (DOM) strongly influences its biogeochemical role in freshwater ecosystems, yet DOM composition measurements are not routinely incorporated into ecological studies. To date, the majority of studies of freshwater ecosystems have relied on bulk analyses of dissolved organic carbon and nitrogen to obtain information about DOM cycling. The problem with this approach is that the biogeochemical significance of DOM can only partially be elucidated using bulk analyses alone because bulk measures cannot detect most carbon and nitrogen transformations. Advances in fluorescence spectroscopy provide an alternative to traditional approaches for characterizing aquatic DOM, and allow for the rapid and precise characterization of DOM necessary to more comprehensively trace DOM dynamics. It is within this context that we discuss the use of fluorescence spectroscopy to provide a novel approach to tackling a long-standing problem: understanding the dynamics and biogeochemical role of DOM. We highlight the utility of fluorescence characterization of DOM and provide examples of the potential range of applications for incorporating DOM fluorescence into ecological studies in the hope that this rapidly evolving technique will further our understanding of the biogeochemical role of DOM in freshwater ecosystems.

Dissolved organic matter (DOM) is one of the largest sources of biologically available organic carbon in aquatic ecosystems and its dynamics have implications for carbon cycling on local to global scales (Battin et al. 2009). Tremendous progress has been made in the past 30 yr toward understanding the biochemical structure of DOM and the role that it plays in the biogeochemistry of aquatic ecosystems. Significant improvements in DOM characterization techniques and molecular biology combined with more interdisciplinary studies across traditional scientific boundaries have all led to the understanding that DOM is a complex mixture of soluble organic compounds that vary in their reactivity and ecological role. Moreover, it is now widely accepted that aquatic DOM is not dominated by heavily processed, refractory material, but rather it plays a critically important role in aquatic food webs because it supplies carbon (Wetzel 1992) and nitrogen (Keil and Kirchman 1991) for heterotrophic production. However, the extent to which these dissolved organic forms of carbon (DOC) and nitrogen (DON) are metabolized in freshwater ecosystems depends largely on the biochemical composition of the DOM in which they are contained (Benner 2003).

Despite the recognition that DOM composition strongly influences its role in the environment, DOM characterization is still not routinely included in many biogeochemical studies (Jaffe et al. 2008). This is particularly apparent in the lack of long-term or broad, cross-site comparison studies that specifically evaluate the biochemical composition of DOM (Dittman et al. 2007; Zhang et al. 2010). To characterize DOM in freshwater ecosystems, researchers have previously focused on bulk properties such as C : N ratios, stable isotope analyses (e.g., ^{13}C and ^{15}N; Hood et al. 2005), and also measurements of amino acids, carbohydrates, and lignin phenols (McDowell and Likens 1988; Spencer et al. 2008). All of these techniques provide useful information about the composition, origin, and function of DOM. However, there are several drawbacks, including large sample volume, relatively complex and time-consuming sample preparation and analyses, and high analytical costs that have prevented them from routine incorporation in long-term or spatially extensive studies. In this context, a major challenge for ecosystem scientists has been to develop straightforward analytical techniques that will improve our understanding of DOM dynamics in aquatic ecosystems.

Advances in spectroscopic techniques (e.g., absorbance and fluorescence) provide an alternative to traditional approaches for characterizing DOM in aquatic ecosystems (Coble et al. 1990; McKnight et al. 2001). The optically active fraction of DOM can trace compositional changes because biochemical characteristics of DOM can be related to its optical properties (Stedmon et al. 2003; Hernes et al. 2009). Fluorescence characterization of DOM provides reliable information about the source, redox state, and biological reactivity of DOM (Miller et al. 2009; Mladenov et al. 2010). This technique is also rapid, precise, and relatively inexpensive to undertake, which allows for the temporal and spatially extensive sampling programs necessary to comprehensively trace DOM dynamics in aquatic ecosystems. For example, a cross-site comparison study of 12 Long-Term Ecological Research (LTER) sites showed that DOM fluorescence characteristics varied dramatically, both temporally and spatially, over the range of physical and ecological conditions (e.g., hydrology and landcover) present at each site (Jaffe et al. 2008).

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Here we assert that fluorescence spectroscopy shows clear potential for elucidating the biogeochemical role of DOM in freshwater environments by enhancing the ability of ecosystem scientists to incorporate routine measurements of DOM quality into their research programs. Our goal is twofold: to provide a broad overview of DOM fluorescence and to foster a wider understanding and appreciation of the merits of fluorescence spectroscopy in the hope that this rapidly evolving technique will become more widely used by the ecological community. We begin by describing common methods for measuring DOM fluorescence and provide definitions of routinely used fluorescence components and indices with the purpose of linking these metrics to ecologically meaningful characteristics of DOM. We then provide examples of studies where fluorescence has been used to elucidate aquatic DOM dynamics and conclude by highlighting several key areas where this technique could greatly assist in future efforts to develop our understanding of how DOM is cycled in aquatic ecosystems.

Fluorescence characterization of DOM

In recent decades, fluorescence spectroscopy has been used to investigate the dynamics of DOM in a range of aquatic environments, and this research has been the focus of recent reviews in marine ecosystems (Blough and Del Vecchio 2002; Nelson and Siegel 2002; Coble 2007), as well as freshwaters and water treatment systems (Hudson et al. 2007; Henderson et al. 2009). To date, studies of marine environments have made important advances in the field of DOM fluorescence, and this legacy is reflected in the large number of marine references in this review. There is substantial overlap in the equipment and analytical methods for DOM fluorescence in freshwater and marine environments. However, fluorescence in marine studies has predominantly focused on autochthonous production and identifying terrestrial plant-derived DOM in marine environments (Coble 1996; Murphy et al. 2008), whereas fluorescence has been used to identify a broad range of natural (e.g., soils and plant litter) and anthropogenic (e.g., wastewater, urban, and agricultural runoff) DOM inputs into freshwater ecosystems.

Fluorescence occurs when a molecule absorbs energy causing an electron to be excited to a higher energy level, and as the electron returns to the original ground state, energy is lost as light or fluorescence. Thus, the excitation and emission wavelengths at which fluorescence occurs are characteristic to specific molecular structures. Organic compounds that absorb (chromophores) and re-emit light are referred to as fluorophores (Mopper et al. 1996). To date, two distinct types of DOM fluorescence groups have been found in aquatic ecosystems (Coble et al. 1990): one has fluorescence properties similar to proteins and the other is similar to broadly defined humic substances (Table 1). Dissolved humic substances typically account for a large portion of the fluorescence occurring in natural waters and compounds such as lignin, tannins, polyphenols, and melanins are likely responsible for the bulk of humic DOM fluorescence (Green and Blough 1994; Del Vecchio and Blough 2004). Quinone moieties have also been suggested to contribute to humic DOM fluorescence and research has shown that $> 50\%$ of DOM fluorescence is potentially due to such structures (Cory and McKnight 2005). Although the exact chemical compounds responsible for DOM fluorescence are still partly undefined, there is much knowledge to be gained about their ecological relevance through tracing their dynamics in the field and laboratory experiments (Baker and Spencer 2004; Cory et al. 2007).

Completing DOM fluorescence measurements is a relatively simple but multiple-step process that requires careful instrument calibration because it is important to correct for instrument-specific bias (Stedmon and Bro 2008). Instrument-specific corrections account for spectral bias of instrument components (e.g., gratings and mirrors), deviations in spectral output of the light source, and calibration of signal intensity by normalizing the DOM fluorescence to quinine sulfate or the Raman signal of pure water (Stedmon et al. 2003; Stedmon and Bro 2008). It is also important to recognize that there are several drawbacks of this technique. For instance, fluorescence characterization of DOM does not provide definitive information on the biochemical structure of DOM or actual concentration of organic compounds, and only a small fraction of the DOM pool likely contributes to its fluorescence. There is potential for shifts in fluorescence characteristics with temperature (Baker 2005), oxygen concentration (Fulton et al. 2004), and extremes of pH (Mobed et al. 1996), and spectral interference of fluorescence due to highly absorbing sample (e.g., inner-filter effects) or metal (e.g., iron) quenching (McKnight et al. 2001). Additionally, there are still problems with spectrofluorometers (e.g., light source wavelength range; Hudson et al. 2007) and techniques for analyzing DOM fluorescence (Stedmon and Bro 2008).

There are a variety of indices used to quantify differences in the fluorescence properties of DOM (Table 1). One of the simplest and most widely used is the two-dimensional fluorescence index (FI), which provides information about the source (e.g., microbial or terrestrial higher plant material) or degree of degradation of DOM because it reflects the relative contribution of aromatic vs. nonaromatic DOM (Table 1; McKnight et al. 2001; Cory and McKnight 2005). Fluorescence DOM measurements are also commonly collected as three-dimensional excitation–emission matrices (EEMs; Coble et al. 1990; McKnight et al. 2001), which are produced from multiple emission spectra collected at successively increasing excitation wavelengths (Fig. 1). The EEMs contain a large amount of information about the composition, origin, and processing of DOM. Diagnostic indices, such as the humification index (HIX; Parlanti et al. 2000; Ohno 2002) and $\beta:\alpha$ values (Parlanti et al. 2000), are commonly used to analyze EEMs (Table 1). These indices use ratios of fluorescence intensity in different regions of the EEM to provide information about the source, degree of humification, and relative contribution of recently produced to structurally complex and aromatic DOM.

Visual inspection of EEMs or ''peak picking'' of fluorescence peaks is also a commonly used technique for

Table 1. Ecological definitions for fluorescence components and indices. Abbreviations are em = emission and ex = excitation.

Parameter	Calculated	Description
Protein-like components	Tyrosine-like and tryptophan-like fluorescence components.	Amino acids, free, bound in proteins, or associated with high-molecular-weight DOM (*see* Baker et al. 2007). Indicator of biologic activity, DOM bioavailability, cycling of fast and slow pools of DOM, and water quality.
Humic-like components	*See* Table 2 for a list of humic-like fluorescence components.	Components that exhibit emission at long wavelengths are thought to be aromatic, contain many conjugated fluorescent molecules, and are red shifted. A blue shift in emission maximum (emission at shorter wavelengths) can be caused by a decrease in the number of aromatic rings, or a reduction of conjugated bonds in a chain structure (Coble 1996).
Fluorescence index (FI)	The ratio of em wavelengths 450 nm and 500 nm, obtained at ex 370 nm (McKnight et al. 2001). However, the FI was modified by Cory and McKnight (2005) to the ratio of em wavelengths at 470 nm and 520 nm, obtained at ex 370.	Determine source of DOM, which is either: microbial (high FI ~1.8, derived from extracellular release and leachate from bacteria and algae) or terrestrially derived (low FI ~1.2, terrestrial plant and soil organic matter).
Freshness index ($\beta:\alpha$)	Ratio of em intensity at 380 nm divided by the em intensity maximum observed between 420 and 435 nm, obtained at ex 310 nm (Parlanti et al. 2000; Wilson and Xenopoulos 2009).	Indicator of the contribution of recently produced DOM, where β represents more recently derived DOM and α represents more decomposed DOM.
Humification index (HIX)	Calculated by Zsolnay et al. (1999) as the peak area under the em spectra 435–480 nm divided by 300–445 nm, at ex 254 nm. Later modified by Ohno (2002) to the area under the em spectra 435–480 nm divided by the peak area 300–345 nm + 435–480 nm, at ex 254 nm.	Indicator of humic substance content or extent of humification. The HIX is based on the idea that the emission spectra of fluorescing molecules will shift toward longer wavelengths (due to lower H:C ratios) as humification of DOM proceeds. Higher values indicate an increasing degree of humification.
Redox index (RI)	$Q_{red}/(Q_{red}+Q_{ox})$, where Q_{red} is the sum of the reduced components and Q_{ox} is the sum of the oxidized components (Miller et al. 2006).	Oxidation state of DOM fluorescence.

analyzing EEMs (Coble et al. 1990; Coble 1996), and the intensity of individual peaks can be compared across a range of sample dates (Baker 2001). Within the large number of natural and anthropogenic fluorophores identified to date (Westerhoff et al. 2001; Coble 2007; Hudson et al. 2007), we focus our discussion on five primary peaks: humic-like peaks A, C, and M and protein-like peaks B and T (Fig. 1; Table 2) because we believe they can be readily linked to ecologically meaningful characteristics of DOM, and have clear potential to become widely used by the ecological community. These fluorescence peaks were originally identified in studies by Coble et al. (1990) and Coble (1996), and have been the basis for fluorescence comparisons in numerous studies over the years. In general, peaks that exhibit emission at long wavelengths (referred to as ''red shifted'') and have broad emission maxima contain many conjugated fluorescence molecules. Peaks A and C are examples of such fluorophores, and are derived primarily from vascular plant sources, aromatic in nature, highly conjugated, and likely represent the higher-molecular-weight fraction of the DOM pool (Coble et al. 1998). In contrast, peak M is slightly ''blue shifted'' because it exhibits emission at shorter wavelengths (~ 370–430 nm) and is thought be less aromatic and of lower molecular weight than peaks A and C. Peak M was originally used as a marker for in situ DOM production because it was thought to be derived exclusively from marine planktonic production, although several studies have identified it in terrestrial and freshwater environments (Stedmon and Markager 2005*a*; Murphy et al. 2008; Balcarczyk et al. 2009). Overall, differences in the contribution of these humic-like fluorescence groups can be used to distinguish between terrigenous (derived from higher plant material) and autochthonous DOM (produced within the aquatic environment).

Over the last decade there has been substantial research evaluating the source and biogeochemical role of the fluorescent amino acid-like peaks tyrosine (peak B) and tryptophan (peak T, Parlanti et al. 2000; Yamashita and Tanoue 2003; Maie et al. 2007). Previous research of coastal marine environments has shown that protein-like fluorescence intensities are correlated with concentrations of tyrosine and tryptophan in total hydrolyzable amino acids (Yamashita and Tanoue 2003). However, tyrosine and tryptophan do not typically occur as pure dissolved amino acids in the environment, as their florescence spectra are seldom identical to pure reference standards (Yamashita and Tanoue 2003; Cory and McKnight 2005). Thus,

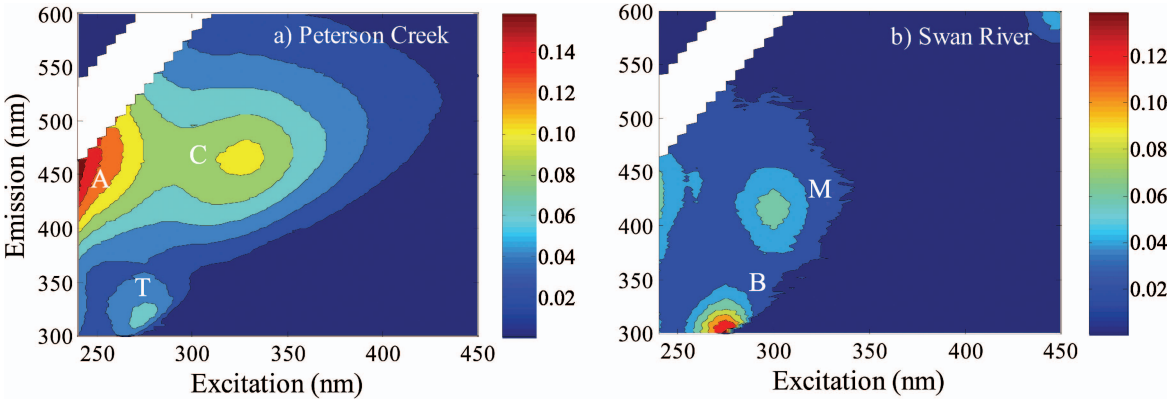

Fig. 1. Example of two riverine EEMs showing the position of the five primary fluorescence peaks in optical space: (a) Peterson Creek, Juneau, Alaska and (b) Swan River, Perth, Australia (Fellman unpubl.). The white area in the upper left corner of each EEM is where excess scatter is removed from the EEM. Fluorescence intensities are in Raman units.

protein-like fluorescence is likely derived from a mixture of dissolved amino acids and other organic materials with similar fluorescence characteristics (Maie et al. 2007). Protein-like components have considerable potential for use as biogeochemical tracers, as they have been used to predict DOM lability (Fellman et al. 2009*a*) and to fingerprint DOM derived from microbial sources (Hood et al. 2009). Although protein-like fluorescence has great potential for use in a range of ecological applications, much still needs to be learned, particularly in freshwater ecosystems with high DOM concentrations, about the molecular size (Maie et al. 2007) and structure of DOM that contributes to protein-like fluorescence (Hernes et al. 2009).

Excitation–emission matrices can also be analyzed using the multivariate modeling technique parallel factor analysis (PARAFAC; Bro 1997; Stedmon et al. 2003; Cory and McKnight 2005), a three-way method that decomposes the fluorescence signature of DOM into individual components and provide estimates of the relative contribution of each component to total DOM fluorescence. Thus, PARAFAC components provide information about the biochemical composition, origin, and biogeochemical role of aquatic DOM. Studies have used PARAFAC analysis of EEMs to characterize DOM from diverse environments ranging from microbially dominated Antarctic lakes (Cory and McKnight 2005) to experimentally acidified forest soils in Maine (Ohno et al. 2007), with as many as 13 components being identified in a single PARAFAC model (Cory and McKnight 2005). To help standardize PARAFAC component identification across studies as well as link components with ecologically meaningful characteristics of DOM,

Table 2. Summary of commonly observed natural fluorescence peaks of aquatic DOM. The selected references represent the original studies that summarized and named specific fluorescence peaks and highlight the differences in nomenclature used.

Component	Excitation and emission maxima (nm)	Peak name	Probable sources*	Description
Tyrosine-like	ex 270–275, em 304–312	B‡, γ§	T, A, M	Amino acids, free or bound in proteins, fluorescence resembles free tyrosine, may indicate more degraded peptide material
Tryptophan-like	ex 270–280 (<240), em 330–368	B†, T‡, δ§	T, A, M	Amino acids, free or bound in proteins, fluorescence resembles free tryptophan, may indicate intact proteins or less degraded peptide material
Ultraviolet A (UVA) humic-like	ex 290–325 (<250), em 370–430	M‡, β§	T, A, M	Low molecular weight, common in marine environments associated with biological activity but can be found in wastewater, wetland, and agricultural environments
UVC humic-like	ex <260, em 448–480	A†, A‡, α'§	T	High molecular weight and aromatic humic, widespread, but highest in wetlands and forested environments
UVC humic-like	ex 320–360, em 420–460	C†, C‡, α§	T	High-molecular-weight humic, widespread, but highest in wetlands and forested environments
Unknown	280/370	N‡	A	Very labile, associated with freshly produced DOM

* T, terrestrial plant or soil organic matter; A, autochthonous production; M, microbial processing; U, unknown.
† Coble et al. (1990).
‡ Coble et al. (1998).
§ Parlanti et al. (2000).

we summarized commonly observed components from the original studies that described and named specific PARAFAC components (Table 3). Fluorescence components are commonly referred to as humic-like, fulvic-like, or protein-like (also referred to as either tyrosine- or tryptophan-like) because each component represents a group of fluorophores with fluorescence characteristics similar to those of reference standards (e.g., Elliot soil standard humic acid, International Humic Substances Society), rather than of a pure fluorophore (Stedmon et al. 2003). The most common approach to obtain PARAFAC component information from EEMs is to build an original model if a large enough number of EEMs are collected (tutorial by Stedmon and Bro 2008).

The benefit of PARAFAC is that a more complete analysis of EEMs is possible than for traditional peak-picking methods and additional information such as oxidation state (redox index [RI]; Table 1) may be obtained from the EEMs. Moreover, PARAFAC can take overlapping fluorescence spectra and decompose them into broadly defined fluorescence components. For example, the humic-like components 1 (excitation [ex] = 240, emission [em] = 476) and 2 (ex = 240, em = 398) of Stedmon and Markager (2005*b*) alone do not have similar fluorescence spectra to peak A. However, their combined spectra have excitation ($<$ 250 nm) and emission maxima ($\sim$ 432 nm) similar to that of peak A. Thus, a different combination of these two components caused the emission maximum to vary between 398 and 476 nm, and is consistent with the large range in emission maxima reported in the literature for individual humic-like components.

Incorporating fluorescence characterization into freshwater ecological studies

Fluorescence characterization has great potential to be more widely incorporated into research on DOM cycling. Here, we provide several contemporary examples of studies where fluorescence characterization has been used as a tool to provide new insights into the biogeochemistry of DOM in freshwater ecosystems. Our intent is to highlight both the utility of this technique and a range of applications for incorporating DOM into ecological studies.

Effect of land use on DOM character and cycling—Fluorescence measurements of DOM have been used to determine the current and future effects of land use and land management practices on stream biogeochemistry (Huang and Chen 2009; Wilson and Xenopoulos 2009; Williams et al. 2010). For example, research on a land use gradient of increasing cropland coverage and decreasing wetland coverage showed an increase in the contribution of recently produced DOM, as indicated by $\beta:\alpha$ values or the "freshness index" (Wilson and Xenopoulos 2009). Moreover, the FI and HIX both indicated that this recently produced DOM was less structurally complex than wetland DOM and was likely derived from microbial sources. Simple fluorescence indices can therefore be used to improve our understanding of how changes in land use may affect the biochemical composition of DOM and aquatic food-web dynamics.

The fluorescence characterization of DOM can also be used to identify water pollution in agricultural and urban catchments (Westerhoff et al. 2001; Baker and Inverarity 2004). For example, research from an urban catchment in the U.K. that receives wastewater inputs showed that protein-like fluorescence was significantly correlated with biological oxygen demand and inorganic N and P concentrations (Baker and Inverarity 2004). With technological advances that now enable in situ collection of fluorescence and absorbance measurements (Spencer et al. 2007; Saraceno et al. 2009), spectroscopic techniques have the potential to be a useful tool for water-quality monitoring in urban and agricultural catchments.

Evaluation of DOM lability and biological activity—Protein-like fluorescence has a range of potential applications in ecological studies through tracing its dynamics in the field or in laboratory experiments (Fellman et al. 2008; Balcarczyk et al. 2009; Lapworth et al. 2009). For example, protein-like fluorescence has been correlated with bacterial production, bacterial respiration, and community respiration in southern Quebec lakes (Cammack et al. 2004). Protein-like fluorescence can also indicate DOM lability, as laboratory incubations of soil water, stream water, and glacier DOM collected from southeastern Alaskan watersheds found a strong relationship between protein-like fluorescence and biodegradable DOC (Fellman et al. 2009*a*; Hood et al. 2009). Furthermore, fluorescence characterization can trace changes in the composition of DOM during biodegradation studies, as Wickland et al. (2007) showed that microbial processing of DOM resulted in an increase in the HIX or degree of humification during a laboratory incubation of moss leachate (Fig. 2).

Assessment of biogeochemical transformations—The RI (*see* Table 1) is a measure of the oxidation state of DOM, and can be used to study redox reactivity in surface water and groundwater (Miller et al. 2006; Mladenov et al. 2008, 2010). For example, a study evaluating nutrient cycling in a wetland–stream ecosystem used the RI to show that DOM in a wetland adjacent to a stream was reduced (Miller et al. 2006). Additionally, dissolved iron concentrations were high and ammonium was the dominant form of inorganic N in the wetland. As water from the wetland entered the stream, there was a shift in the RI as DOM was rapidly oxidized, ammonium was converted to nitrate, and dissolved iron concentrations decreased. Thus, fluorescence can provide information about the environmental conditions and potential biogeochemical transformations that are occurring, particularly in studies evaluating nutrient cycling in hyporheic or riparian ecosystems.

In-stream uptake of DOM fractions—The relationship between humic-like and protein-like fluorescence components can assist in determining the potential fate or biological uptake of DOM and its different fractions by stream ecosystems. Fellman et al. (2009*b*) showed that protein-like fluorescence decreased downstream during soil leachate additions in forested headwater streams, whereas humic-like fluorescence did not change. These observed

Table 3. Summary of commonly observed fluorescence PARAFAC components of aquatic DOM (only includes natural organic compounds that fluoresce). The selected references represent the original studies that summarized and named specific fluorescence components.

Component	Excitation and emission maxima (nm)	PARAFAC component no.	Probable sources*	Description
Tyrosine-like	ex 270–275, em 304–312	13‡, 8§, 1‖	T, A, M	*See* Table 2 above for description
Tryptophan-like	ex 270–280 (<240), em 330–368	5†, 8‡, 7§, 6‖	T, A, M	*See* Table 2 above for description
Protein-like	ex 240 (300), em 338	7‖	T, A, M	Amino acids, free or bound in proteins, fluorescence resembles free tryptophan
UVA humic-like	ex 290–325 (<250), em 370–430	4†, 3‡, 5§, 2‖	T, A, M	Low molecular weight, common in marine environments associated with biological activity but can be found in wastewater, wetland, and agricultural environments
UVA humic-like	ex 250–295 (360–385), em 478–504	3†, 5‡, 2§, 3‖	T	High molecular weight, aromatic, fluorescence resembles fulvic acid, widespread
UVA humic-like	ex <260, em 440	11‡, 4§	T	Fluorescence resembles fulvic acid, widespread
UVC humic-like	ex <260, em 448–480	1†, 2‡, 1§, 8‖	T	High molecular weight and aromatic humic, widespread, but highest in wetlands and forested environments
UVC humic-like	ex <250 (305), em 412–416	2†, 3§	T	High-molecular-weight humic, widespread, but highest in wetlands and forested environments
Humic-like	ex <250 (320), em 400	6§	T	Common in wastewater and agricultural catchments
UVC humic-like	ex 320–360, em 420–460	1‡	T	High-molecular-weight humic, widespread, but highest in wetlands and forested environments
Unknown	ex 250 (320), em 370	4‖	U	Possible polycyclic aromatic hydrocarbon
Humic-like	ex 250, em 550	4‡	T, M	Reduced, humic-like, correlated with % anomeric, acetal and ketal C
Unknown	ex 270, em 430	6‡	M	Unknown humic-like fluorescence group
Humic-like	ex 270 (380), em 462	7‡	M	Reduced, humic-like
Humic-like	ex 345 (265), em 410	9‡	M	Reduced, humic-like
Unknown	ex 305, em 426	10‡	T	Unknown humic-like fluorescence group
Humic-like	ex <250, em 388–425	12‡, 9‖	A, M	Oxidized, humic-like, correlated with aliphatic C content, associated with autochthonous production, potential photoproduct of terrestrial DOM

* T, terrestrial plant or soil organic matter; A, autochthonous production; M, microbial processing; U, unknown.
† Stedmon et al. (2003).
‡ Cory and McKnight (2005).
§ Stedmon and Markager (2005*a*).
‖ Murphy et al. (2006).

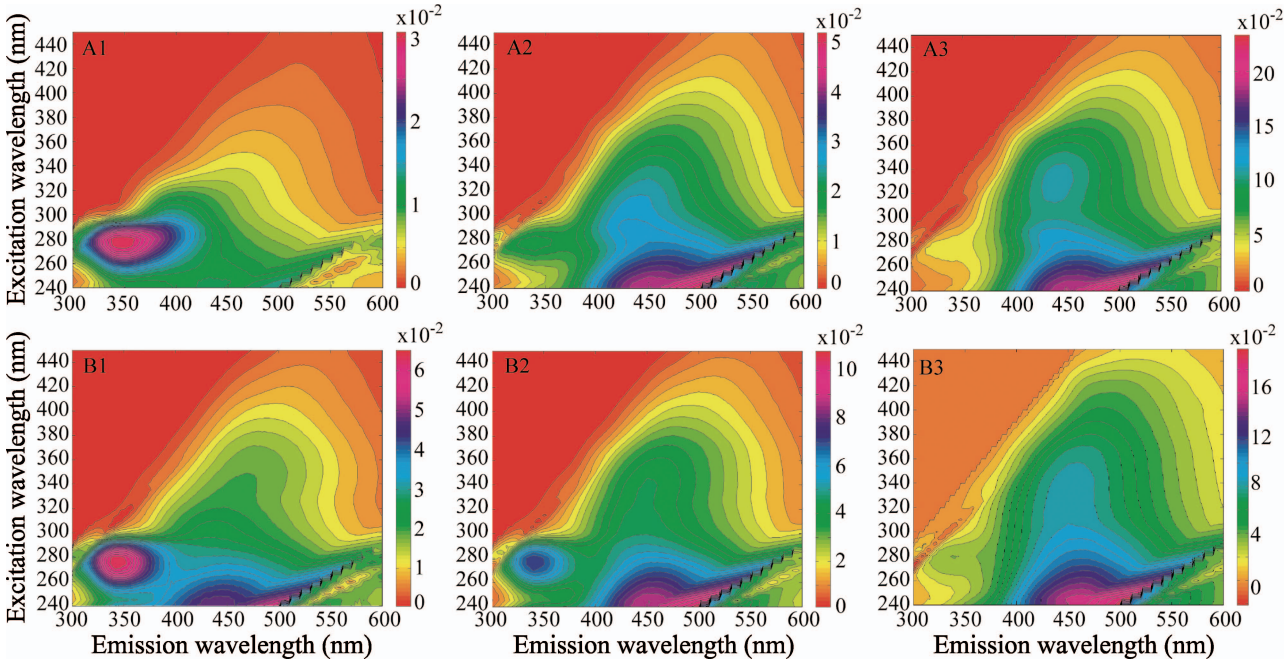

Fig. 2. Change in fluorescence of moss leachate DOM over time for (A1–A3) *Sphagnum angustifolium* and (B1–B3) Feathermoss mix at 24 h, 194 h, and 3 months (reprinted from Wickland et al. 2007). Tryptophan-like fluorescence (ex = 275, em = 346) was the dominant component in both leachates after 24 h (A1 and B1). After 3 months, the percent contribution of tryptophan-like fluorescence decreased 83% and 97% for the *Sphagnum* and Feathermoss leachate, respectively. Fluorescence intensities are in Raman units normalized to DOC concentration.

changes indicate that certain fractions of DOM were selectively removed while others remain. Moreover, protein-like fluorescence was removed at a significantly greater rate than bulk concentrations of DOC and DON, consistent with the idea of different pools comprising DOM turnover at different rates (Brookshire et al. 2005). Fluorescence characterization of DOM combined with traditional in-stream tracer releases to measure DOM uptake could therefore help elucidate the role of DOM pools of varying reactivity in supporting heterotrophic metabolism in aquatic ecosystems.

Effects of photochemical processes on DOM character and cycling—Fluorescence characterization of DOM can also show how photochemical processes induce changes in DOM composition (Moran et al. 2000; Cory et al. 2007; Stedmon et al. 2007). For example, photodegradation studies of Alaskan stream and lake water showed a large decrease in fluorescence for aromatic, humic-like components, which resulted in an increased contribution of protein-like relative to humic-like components after exposure to light (Cory et al. 2007). Thus, fluorescence can help understand how photochemical processes influence DOM bioavailability, the penetration of ultraviolet and photosynthetically active wavelengths of light (Vodacek et al. 1997), and the fate of terrigenous DOM in the ocean (Spencer et al. 2009).

Indicator of temporal variation in DOM source and character—The relationship between humic-like and protein-like fluorescence is useful for studies aimed at identifying different sources that contribute to stream DOM (Hood et al. 2007; Jaffe et al. 2008; Yamashita et al. 2010). For example, research in an Alaskan salmon spawning stream showed that DOM derived from salmon carcasses is rich in protein-like fluorescence, and that salmon-derived DOM is chemically distinct from the humic-rich, wetland-derived DOM that dominates the aquatic load during the nonspawning period (Fig. 3; Hood et al. 2007). Thus, fluorescence spectroscopy can fingerprint ephemeral inputs into the bulk pool of stream DOM.

Another application for humic-like and protein-like fluorescence is for evaluating seasonal patterns in the source and composition of DOM (Jaffe et al. 2008; Fellman et al. 2009*b*; Miller and McKnight 2010). Jaffe et al. (2008) showed that protein-like fluorescence reached its highest relative abundance during the summer in Florida Bay, indicating that primary production is responsible for the peak in protein-like fluorescence (Fig. 4). However, humic-like components derived from catchment sources of plant and soil organic matter peaked during the fall months when maximum water discharge from the Everglades commonly occurs. Therefore, the observed seasonal pattern in the contribution of protein-like and humic-like components suggests that humic-like fluorescence is strongly controlled by hydrological processes, whereas protein-like fluorescence is more tightly linked with biological processes.

Future directions: Opening up the DOM black box

There is still a large need for fundamental information about the source, biochemical composition, and fate of

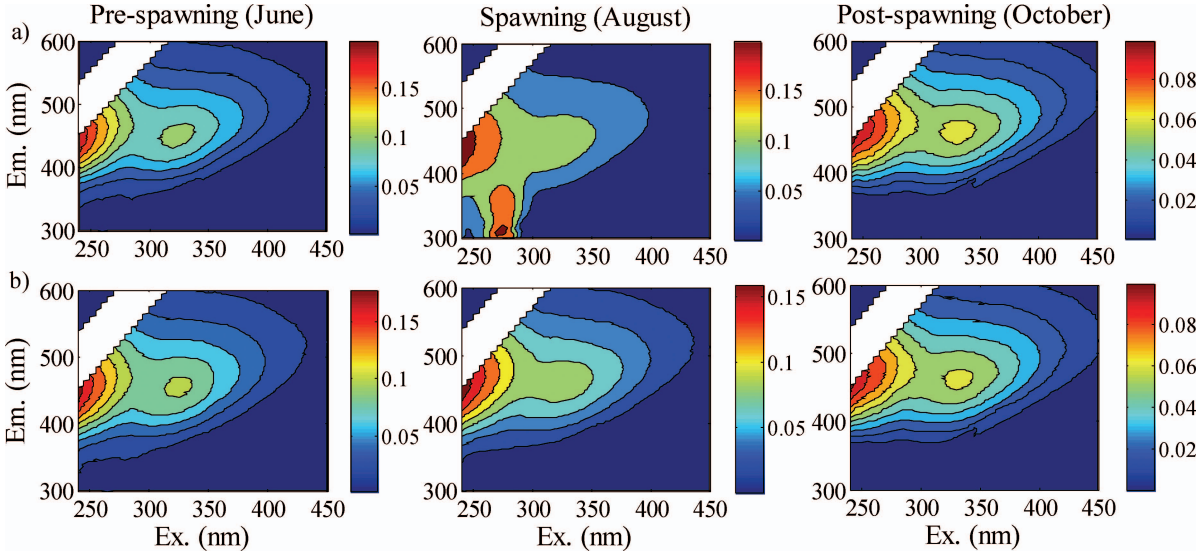

Fig. 3. The EEMs for DOM before, during, and after the salmon spawning period at the (a) Lower and (b) Upper Peterson Creek sites (reprinted from Hood et al. 2007). Before and after the spawning period, EEMs at both the upstream and downstream sites showed a primary peak at an excitation of 240 nm and an emission of 450 nm. During the spawning period, the EEM at the Lower Peterson site with spawners contained an additional peak at an excitation of 275 nm and an emission of 308 nm. Fluorescence intensities are in Raman units.

DOM in ecosystems. Here we identify several key areas of research where fluorescence spectroscopy could enhance our understanding of the role DOM fills in freshwater ecosystems. There is increasing interest in how present-day and future anthropogenic and climatic drivers will influence the dynamics of aquatic ecosystems. Thus, fluorescence characterization of DOM should be included as part of routine biogeochemical analysis in long-term watershed monitoring sites, such as at LTER sites or in large cross-site comparison studies such as the Lotic Intersite Nitrogen Experiments 1 and 2. Long-term monitoring of DOM composition will improve understanding of and predictions about how anthropogenic and climatic perturbations will affect the metabolic stability of freshwater and downstream marine ecosystems. This, in turn, will lead to more successful ecosystem restoration and improved watershed-scale management strategies.

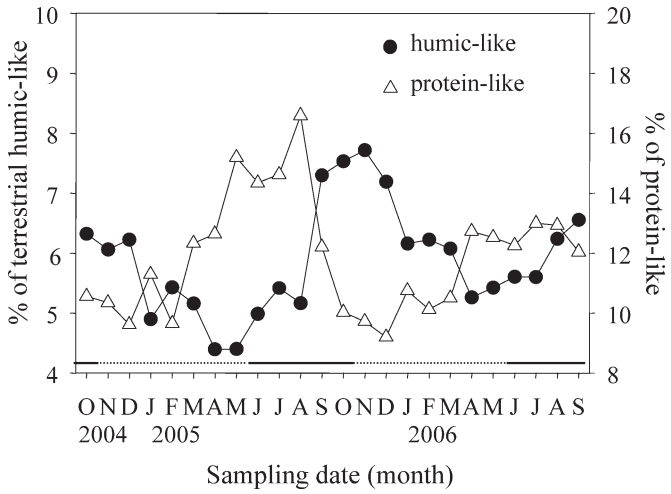

Fig. 4. Seasonal variability in the percent contribution of protein-like and humic-like fluorescence as determined from PARAFAC analysis of EEMs (reprinted from Jaffe et al. 2008).

Much still needs to be learned about the biogeochemical role of DON in aquatic ecosystems, in part because the analysis of DON composition still lags behind DOC. It is now clear that DON plays an important role in terrestrial N cycling (Neff et al. 2003) and as new studies examine the cycling of DON in aquatic ecosystems, it will be important to develop a more complex view of DON as a mixture of labile and recalcitrant compounds that have substantial and very different roles (Brookshire et al. 2005). In this context, tracing the dynamics of DOM fluorescence, particularly protein-like components (e.g., tyrosine- and tryptophan-like), could help elucidate the relative role of readily available and recalcitrant pools of both DOC and DON in supporting stream metabolism.

Understanding the cycling of DOM in freshwater ecosystems is ultimately linked to the development of analytical techniques and our ability to relate DOM composition to biological measurements. Fluorescence characterization is a relatively simple and effective ecological tool that can readily be used in spatially and temporally extensive sampling programs (Jaffe et al. 2008). Thus, we argue that DOM fluorescence should become embedded in ecological studies such that it becomes a more routine water-quality metric, similar to that of DOC and inorganic nutrient concentrations. Methods for fluorescence DOM characterization combined with PARAFAC are also becoming more standardized and widely available (tutorial by Stedmon and Bro 2008). Moreover, the potential for in situ monitoring of DOM fluorescence will allow for high-resolution collection of data in the field (Spencer et al. 2007; Saraceno et al. 2009). With the recent technological advances and the availability of bench-top spectrofluorom-

eters that provide rapid generation of EEMs, the Achilles heel of incorporating DOM composition measurements into ecological studies should no longer be methodological. Rather, the main challenges lie in effectively utilizing emerging techniques such as fluorescence spectroscopy to further our understanding of the biogeochemical role of DOM in aquatic ecosystems.

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References

Baker, A. 2001. Fluorescence excitation–emission matrix characterization of some sewage-impacted rivers. Environ. Sci. Technol. **35:** 948–953, doi:10.1021/es000177t

———. 2005. Thermal fluorescence quenching properties of dissolved organic matter. Water Res. **39:** 4405–4412.

———, and R. Inverarity. 2004. Protein-like fluorescence intensity as a possible tool for determining river water quality. Hydrol. Process. **18:** 2927–2945, doi:10.1002/hyp.5597

———, and R. G. M. Spencer. 2004. Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy. Sci. Total Environ. **333:** 217–232, doi:10.1016/j.scitotenv.2004.04.013

Balcarczyk, K. L., J. B. Jones, Jr., R. Jaffe, and N. Maie. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. Biogeochemistry **94:** 255–270, doi:10.1007/s10533-009-9324-x

Battin, Y. J., S. Luyssaert, L. Kaplan, A. K. Aufdenkampe, A. Richter, and L. J. Tranvik. 2009. The boundless carbon cycle. Nature Geosci. **2:** 598–600, doi:10.1038/ngeo618

Benner, R. 2003. Molecular indicators of the bioavailability of dissolved organic matter, p. 121–137. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], Aquatic ecosystems: Interactivity of dissolved organic matter. Elsevier.

Blough, N. V., and R. Del Vecchio. 2002. Chromophoric DOM in the coastal environment, p. 509–546. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Academic.

Bro, R. 1997. PARAFAC. Tutorial and applications. Chemom. Intell. Lab. Syst. **38:** 149–171, doi:10.1016/S0169-7439(97)00032-4

Brookshire, E. N., J. H. M. Valett, S. A. Thomas, and J. R. Webster. 2005. Coupled cycling of dissolved organic nitrogen and carbon in a forest stream. Ecology **86:** 2487–2496, doi:10.1890/04-1184

Cammack, W. K. L., J. Kalff, Y. T. Prairie, and E. M. Smith. 2004. Fluorescent DOM in lakes: Relationships with heterotrophic metabolism. Limnol. Oceanogr. **49:** 2034–2045, doi:10.4319/lo.2004.49.6.2034

Coble, P. G. 1996. Characterization of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy. Mar. Chem. **51:** 325–346, doi:10.1016/0304-4203(95)00062-3

———. 2007. Marine optical biogeochemistry: The chemistry of ocean color. Chem. Rev. **107:** 402–418, doi:10.1021/cr050350+

———, C. E. Del Castillo, and B. Avril. 1998. Distribution and optical properties of CDOM in the Arabian Sea during the 1995 SW monsoon. Deep-Sea Res. II **45:** 2195–2223, doi:10.1016/S0967-0645(98)00068-X

———, S. A. Green, N. V. Blough, and R. B. Gagosian. 1990. Characterization of DOM in the Black Sea by fluorescence spectroscopy. Nature **348:** 432–435, doi:10.1038/348432a0

Cory, R. M., and D. M. McKnight. 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in DOM. Environ. Sci. Technol. **39:** 8142–8149, doi:10.1021/es0506962

———, ———, Y. P. Chin, P. Miller, and C. L. Jaros. 2007. Chemical characteristics of fulvic acids from Arctic surface waters: Microbial contributions and photochemical transformations. J. Geophys. Res. **112:** G04S51, doi:10.1029/2006JG000343

Del Vecchio, R., and N. V. Blough. 2004. On the origin of the optical properties of humic substances. Environ. Sci. Technol. **38:** 3885–3891, doi:10.1021/es049912h

Dittman, J. A., C. T. Driscoll, P. M. Groffman, and T. J. Fahey. 2007. Dynamics of nitrogen and DOC at the Hubbard Brook experimental forest. Ecology **88:** 1153–1166, doi:10.1890/06-0834

Fellman, J. B., D. V. D'Amore, E. Hood, and R. D. Boone. 2008. Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. Biogeochemistry **88:** 169–184, doi:10.1007/s10533-008-9203-x

———, E. Hood, D. V. D'Amore, R. T. Edwards, and D. White. 2009*a*. Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate watersheds. Biogeochemistry **95:** 277–293, doi:10.1007/s10533-009-9336-6

———, ———, R. T. Edwards, and J. B. Jones. 2009*b*. Uptake of allochthonous DOM from soil and salmon in coastal temperate rainforest streams. Ecosystems **12:** 747–759, doi:10.1007/s10021-009-9254-4

Fulton, J. R., D. M. McKnight, C. M. Foreman, R. M. Cory, C. A. Stedmon, and E. Blunt. 2004. Changes in fulvic acid redox state through the oxycline of a permanently ice-covered Antarctic lake. Aquat. Sci. **66:** 27–46, doi:10.1007/s00027-003-0691-4

Green, S. A., and N. V. Blough. 1994. Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. Limnol. Oceanogr. **39:** 1903–1916, doi:10.4319/lo.1994.39.8.1903

Henderson, R. K., A. Baker, K. Murphy, A. Hambly, R. M. Stuetz, and S. J. Khan. 2009. Fluorescence as a potential monitoring tool for recycled water systems: A review. Water Res. **43:** 863–881, doi:10.1016/j.watres.2008.11.027

Hernes, P. J., B. A. Bergamaschi, R. S. Eckard, and R. G. M. Spencer. 2009. Fluorescence-based proxies for lignin in freshwater dissolved organic matter. J. Geophys. Res. **114:** G00F03, doi:10.1029/2009JG000938

Hood, E., J. B. Fellman, and R. T. Edwards. 2007. Salmon influences on dissolved organic matter in a coastal temperate brown-water stream. Limnol. Oceanogr. **52:** 1580–1587.

———, ———, R. G. M. Spencer, P. J. Hernes, R. T. Edwards, D. V. D'Amore, and D. Scott. 2009. Glaciers as a source of ancient and labile organic matter to the marine environment. Nature **462:** 1044–1048, doi:10.1038/nature08580

———, M. W. Williams, and D. M. McKnight. 2005. Sources of DOM in a Rocky Mountain stream using chemical fractionation and stable isotopes. Biogeochemistry **74:** 231–255, doi:10.1007/s10533-004-4322-5

Huang, W., and R. F. Chen. 2009. Sources and transformations of chromophoric DOM in the Neponset River Watershed. J. Geophys. Res. **114:** G00F05, doi:10.1029/2009JG000976

Hudson, N., A. Baker, and D. Reynolds. 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—A review. River Res. Applic. **23:** 631–649, doi:10.1002/rra.1005

Jaffe, R., D. M. McKnight, N. Maie, R. M. Cory, W. H. McDowell, and J. L. Campbell. 2008. Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. J. Geophys. Res. **113:** G04032, doi:10.1029/2008JG000683

Keil, R. G., and D. L. Kirchman. 1991. Contribution of dissolved amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. Mar. Ecol. Prog. Ser. **73:** 1–10, doi:10.3354/meps073001

Lapworth, D. J., D. C. Gooddy, D. Allen, and G. H. Old. 2009. Understanding groundwater, surface water, and hyporheic zone biogeochemical processes in a chalk catchment using fluorescence properties of dissolved and colloidal organic matter. J. Geophys. Res. **114:** G00F02, doi:10.1029/2009JG000921

Maie, N., N. M. Scully, O. Pisani, and R. Jaffe. 2007. Composition of a protein-like fluorophore of DOM in coastal wetland and estuarine ecosystems. Water Res. **41:** 563–570, doi:10.1016/j.watres.2006.11.006

McDowell, W. H., and G. E. Likens. 1988. Origin, composition, and flux of dissolved organic carbon in the Hubbard Brook Valley. Ecol. Monogr. **58:** 177–195, doi:10.2307/2937024

McKnight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Andersen. 2001. Spectrofluorometric characterization of DOM for indication of precursor material and aromaticity. Limnol. Oceanogr. **46:** 38–48, doi:10.4319/lo.2001.46.1.0038

Miller, M. P., and D. M. McKnight. 2010. Comparison of seasonal changes in fluorescent dissolved organic matter among aquatic lake and stream sites in the Green Lakes Valley. J. Geophys. Res. **115:** G00F12, doi:10.1029/2009JG000985

———, ———, S. C. Chapra, and M. W. Williams. 2009. A model of degradation and production of three pools of DOM in an alpine lake. Limnol. Oceanogr. **54:** 2213–2227.

———, ———, R. M. Cory, M. W. Williams, and R. L. Runkel. 2006. Hyporheic exchange and fulvic acid redox reactions in an alpine stream/wetland ecosystem, Colorado Front Range. Environ. Sci. Technol. **40:** 5943–5949, doi:10.1021/es060635j

Mladenov, N., P. Huntsman-Mapila, P. Wolski, R. Wellington, L. Masamba, and D. M. McKnight. 2008. Dissolved organic matter accumulation, reactivity, and redox state in ground water of a recharge wetland. Wetlands **28:** 747–759, doi:10.1672/07-140.1

———, and others. 2010. Dissolved organic matter sources and consequences for iron and arsenic mobilization in Bangladesh aquifers. Environ. Sci. Technol. **44:** 123–128, doi:10.1021/es901472g

Mobed, J. J., S. L. Hemmingsen, J. L. Autry, and L. B. McGown. 1996. Fluorescence characterization of IHSS humic substances: Total luminescence spectra with absorbance correction. Environ. Sci. Technol. **30:** 3061–3065, doi:10.1021/es960132l

Mopper, K., Z. Feng, S. B. Bentjen, and R. F. Chen. 1996. Effects of cross-flow filtration on the absorption and fluorescence properties of seawater. Mar. Chem. **55:** 53–74, doi:10.1016/S0304-4203(96)00048-5

Moran, M. A., W. M. Sheldon, Jr., and R. G. Zepp. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. Limnol. Oceanogr. **45:** 1254–1264, doi:10.4319/lo.2000.45.6.1254

Murphy, K. R., G. M. Ruiz, W. T. M. Dunsmuir, and T. D. Waite. 2006. Optimized parameters for fluorescence-based verification of ballast water exchange by ships. Environ. Sci. Technol. **40:** 2357–2362, doi:10.1021/es0519381

———, C. A. Stedmon, T. D. Waite, and G. M. Ruiz. 2008. Distinguishing between terrestrial and autochthonous organic matter sources in marine environments using fluorescence spectroscopy. Mar. Chem. **108:** 40–58, doi:10.1016/j.marchem.2007.10.003

Neff, J. C., F. S. Chapin, III, and P. M. Vitousek. 2003. Breaks in the cycle: Dissolved organic nitrogen in terrestrial ecosystems. Front. Ecol. **1:** 205–211, doi:10.1890/1540-9295(2003)001[0205:BITCDO]2.0.CO;2

Nelson, N. B., and D. A. Siegel. 2002. Chromophoric DOM in the open ocean, p. 547–578. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Academic.

Ohno, T. 2002. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. Environ. Sci. Technol. **36:** 742–746, doi:10.1021/es0155276

———, I. J. Fernandez, S. Hiradate, and J. F. Sherman. 2007. Effects of soil acidification and forest type on water soluble organic matter properties. Geoderma **140:** 176–187, doi:10.1016/j.geoderma.2007.04.004

Parlanti, E., K. Worz, L. Geoffroy, and M. Lamotte. 2000. Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. Org. Geochem. **31:** 1765–1781, doi:10.1016/S0146-6380(00)00124-8

Saraceno, J. F., B. A. Pellerin, B. D. Downing, E. Boss, P. A. M. Bachand, and B. A. Bergamashi. 2009. High-frequency in situ optical measurements during a storm event: Assessing relationships between DOM, sediment concentrations, and hydrologic processes. J. Geophys. Res. **114:** G00F09, doi:10.1029/2009JG000989

Spencer, R. G. M., G. R. Aiken, K. P. Wickland, R. G. Striegl, and P. J. Hernes. 2008. Seasonal and spatial variability in dissolved organic matter quantity and composition from the Yukon River basin, Alaska. Glob. Biogeochem. Cyc. **22:** GB4002, doi:10.1029/2008GB003231

———, and others. 2007. Diurnal variability in riverine dissolved organic matter composition determined by in situ optical measurement in the San Joaquin River (California, USA). Hydrol. Process. **21:** 3181–3189, doi:10.1002/hyp.6887

———, and others. 2009. Photochemical degradation of dissolved organic matter and dissolved lignin phenols from the Congo River. J. Geophys. Res. **114:** G03010, doi:10.1029/2009JG000968

Stedmon, C. A., and R. Bro. 2008. Characterizing DOM fluorescence with PARAFAC: A tutorial. Limnol. Oceanogr.: Methods **6:** 572–579.

———, and S. Markager. 2005*a*. Resolving the variability in DOM fluorescence in a temperate estuary and its catchment using PARAFAC. Limnol. Oceanogr. **50:** 686–697, doi:10.4319/lo.2005.50.2.0686

———, and ———. 2005*b*. Tracing the production and degradation of autochthonous fractions of DOM fluorescence analysis. Limnol. Oceanogr. **50:** 1415–1426, doi:10.4319/lo.2005.50.5.1415

———, ———, and R. Bro. 2003. Tracing DOM in aquatic environments using a new approach to fluorescence spectroscopy. Mar. Chem. **82:** 239–254, doi:10.1016/S0304-4203(03)00072-0

———, ———, L. Tranvik, L. Kronberg, T. Slatis, and W. Martinsen. 2007. Photochemical production of ammonium and transformation of dissolved organic matter in the Baltic Sea. Mar. Chem. **104:** 227–240, doi:10.1016/j.marchem.2006.11.005

Vodacek, A., N. A. Blough, M. D. Degrandpre, E. T. Peltzer, and R. K. Nelson. 1997. Seasonal variation of CDOM and DOC in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. Limnol. Oceanogr. **42:** 674–686, doi:10.4319/lo.1997.42.4.0674

Westerhoff, P., W. Chen, and M. Esparza. 2001. Fluorescence analysis of a standard fulvic acid and tertiary treated wastewater. J. Environ. Qual. **30:** 2037–2046, doi:10.2134/jeq2001.2037

Wetzel, R. G. 1992. Gradient-dominated ecosystems: Sources and regulatory functions of dissolved organic matter in freshwater ecosystems. Hydrobiologia **229:** 181–198.

Wickland, K. P., J. C. Neff, and G. R. Aiken. 2007. DOC in Alaskan boreal forests: Sources, chemical characteristics, and biodegradability. Ecosystems **10:** 1323–1340, doi:10.1007/s10021-007-9101-4

Williams, C. J., Y. Yamashita, H. F. Wilson, R. Jaffé, and M. A. Xenopoulos. 2010. Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems. Limnol. Oceanogr. **55:** 1159–1171, doi:10.4319/lo.2010.55.3.1159

Wilson, H. F., and M. A. Xenopoulos. 2009. Effects of agricultural land use on the composition of fluvial dissolved organic matter. Nature Geosci. **2:** 37–41, doi:10.1038/ngeo391

Yamashita, Y., N. Maie, H. Briceno, and R. Jaffe. 2010. Optical characterization of dissolved organic matter in tropical rivers of the Guayana Shield, Venezuela. J. Geophys. Res. **115:** G00F10, doi:10.1029/2009JG000987

———, and E. Tanoue. 2003. Chemical characterization of protein-like fluorophores in DOM in relation to aromatic amino acids. Mar. Chem. **82:** 255–271, doi:10.1016/S0304-4203(03)00073-2

Zhang, J., and others. 2010. Long-term patterns in DOC in lakes across eastern Canada: Evidence of a pronounced climate effect. Limnol. Oceanogr. **55:** 30–42, doi:10.4319/lo.2010.55.5.1912

Zsolnay, A., E. Baigar, M. Jimenez, B. Steinweg, and F. Saccomandi. 1999. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere **38:** 45–50, doi:10.1016/S0045-6535(98)00166-0

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