Using in-situ optical sensors to study dissolved organic carbon dynamics of streams and watersheds: A review

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Highlights:

- DOC is a master water quality parameter.
- Mechanisms of DOC processing are inadequately quantified in riverine environments.
- In-situ optical sensors can enhance DOC studies via high-resolution temporal data.
- We discuss these new sensors, their uses, limitations, and future considerations.
- We review and highlight new research directions revealed by these optical sensors.

Keywords: UV-VIS; FDOM; spectroscopy; high-resolution temporal data; rivers

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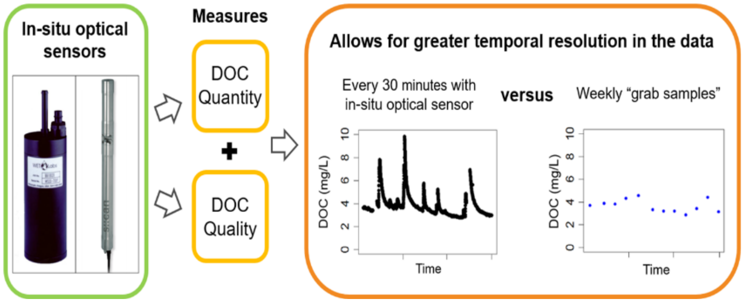
Graphical Abstract



Abstract

It is important to understand how dissolved organic carbon (DOC) is processed and transported through stream networks because DOC is a master water quality variable in aquatic ecosystems. High-frequency sampling is necessary to capture important, rapid shifts in DOC source, concentration, and composition (i.e. quality) in streams. Until recently, this high-frequency sampling was logistically difficult or impossible. However, this type of sampling can now be conducted using in-situ optical measurements through long-term, field-deployable fluorometers and spectrophotometers. The optical data collected from these instruments can quantify both DOC concentration and composition properties (e.g., specific ultra-violet absorbance at 254 nm, spectral slope ratio, and fluorescence index). Previously, the use of these sensors was limited to a small number of specialized users, mainly in Europe and North America, where they were used predominantly in marine DOC studies as well as water treatment and management infrastructure. However, recent field demonstrations across a wide range of river systems reveals a large potential for the use of these instruments in freshwater environments, heightening interest and demand across multiple environmental research and management disciplines. Hence, this review provides an up-to-date synthesis on 1) the use of spectroscopy as a diagnostic tool in stream DOC studies, 2) the instrumentation, its applications, potential limitations and future

considerations, and 3) the new watershed DOC research directions made possible via these in-situ optical sensors.

1. Introduction

Rivers were traditionally thought to play an insignificant role in the global carbon (C) cycle (Battin et al., 2008). However, recent studies reveal that rivers globally receive 4 petagrams (Pg) of terrestrial organic C and generate 1.8 ± 0.25 Pg C as carbon dioxide (CO_2) each year; a rate 6 times greater than lakes and reservoirs combined (Battin et al., 2008; Raymond et al., 2013). This indicates that rivers are a significant source of CO_2 to the atmosphere and that most of the organic C entering rivers is undergoing significant in-stream biogeochemical processing (Battin et al., 2008). Thus, efforts must be made to better understand C cycling in rivers, so that rivers can be considered in future global C models. Dissolved organic carbon (DOC) is often the dominant form of organic C in streams and plays several critical roles in aquatic ecosystems. DOC fuels stream metabolism, regulates additional biogeochemical cycles (e.g., nitrogen transformations), and impacts trace metal complexation and transport (Cory et al., 2011; Aiken, 2014). As a result, stream DOC dynamics influence C cycling on local to global scales (Battin et al., 2009). Therefore, it is important to measure and identify how DOC is processed (e.g., rates, transformations) and transported (e.g., its sources, pathways, and quantity) in riverine environments.

DOC is composed of a complex grouping of molecules with varying chemical structures (e.g., molecular weight and aromaticity). The chemical structure of DOC is a determining factor of DOC quality and is largely dependent on its source material (i.e. allochthonous versus autochthonous) (Cory et al., 2011). Several optical properties of DOC, determined via absorbance and fluorescence spectroscopy, can be used to assess the chemical structure of DOC, which in turn can be used to infer DOC qualities and bioavailability. Ultraviolet-visible (UV-VIS) absorbance spectroscopy can be used to infer aromaticity and molecular weight (e.g., Cory et al., 2011; Jollymore et al., 2012), while fluorescence spectroscopy provides insight on redox state, reactivity, and source material (e.g., Fellman et al., 2010; Cory et al., 2011). DOC quality is a critical parameter that must be included in DOC export studies as the quality of DOC impacts its bioavailability and reactivity (i.e. lability), which ultimately affects downstream ecosystems and water quality (Fellman et al., 2010; Cory et al., 2011).

Until recently, optical measurements of DOC were done using field water collections (i.e. "grab samples") that were later analyzed in the laboratory (e.g., Hood et al., 2006; Spencer et al., 2009; Inamdar et al., 2011). Utilizing lab measurements of optical properties to assess DOC quality is quick, while still maintaining high analytical precision, and relatively inexpensive when compared to chemical analyses (Fellman et al., 2010). However, there is a lack of temporal resolution in the data from using this method, because "grab samples" are often collected at low frequencies. Recent studies have revealed that higher temporal resolution of DOC measurements allows for better understanding of DOC flux and C budgets (e.g., Saraceno et al., 2009; Jollymore et al., 2012; Pellerin et al., 2012; Wilson et al., 2013; Jones et al., 2014; Grayson and Holden, 2016), and allows for further exploration of new ecological theories and scales of processes (e.g., Pulse-Shunt Concept, Raymond et al., 2016). Thus, there is a growing demand for high-quality and cost effective, high-frequency measurements of DOC quantity and quality in riverine environments. A potential solution to this demand are the emerging optically-based field sensor technologies (i.e. long-term, field-deployable fluorometers and spectrophotometers such as the WET Labs, WETStar FDOM Fluorometer and s::can, spectro::lyser), because they have data collection frequencies of minutes. This high-frequency sampling helps to capture changes in both DOC quantity and quality as the result of shifting pathways and sources (e.g., during storm events when it is difficult to manually collect “grab samples”) as well as changes due to in-stream biogeochemical transformations at scales that otherwise would not be seen (e.g., sub-diurnal dynamics, diurnal fluctuations, intra-seasonal trends; Spencer et al., 2007; Pellerin et al., 2012).

These in-situ optically-based field instruments have previously been used by a limited group of specialized users, largely in Europe and North America. The focus of these earlier uses was on DOC dynamics in marine and coastal environments (e.g., Klinkhammer et al., 1997; Chen et al., 1999; Chen and Gardner, 2004; Kowalczuk et al., 2010; Gueguen et al., 2012; Etheridge et al., 2014a; Etheridge et al., 2014b) as well as in water treatment and management infrastructure (e.g., Langergraber et al., 2004; Brinkman and Hozalski, 2011; Boënne et al., 2014; Graham et al., 2015). However, recent riverine field demonstrations reviewed in detail in Section 4 have heightened demand for these optical sensors in many freshwater disciplines, including watershed ecology, biogeochemistry and hydrology (e.g., Spencer et al., 2007; Saraceno et al., 2009; Pellerin et al., 2012).

Despite this growing interest and demand, there is no comprehensive synthesis of the state of the science on using these new technologies (i.e. in-situ optical sensors) in the field. Consequently, potential new users face a daunting task of compiling this information from a wide range of sources (e.g., primary literature to government reports), which inhibits the potential adoption and applications of these new technologies. Consequently, we provide a review on in-situ optical methods used for studying DOC dynamics in riverine environments, specifically via long-term, field-deployable spectrometers and fluorometers. This review will cover 1) what we can learn about DOC from the use of optical properties 2) why in-situ, high-frequency measurements are needed, 3) the instrumentation, its diagnostic uses, limitations, and future considerations, and 4) an illustrative synthesis of future watershed DOC research directions discovered through recent field demonstrations of these instruments.

2. Spectroscopy as a Diagnostic Tool for DOC

Below we briefly review how absorbance and fluorescence spectroscopy is used for assessing DOC composition, and in turn DOC quality, across a wide range of natural waters (McKnight et al., 2001; Belzile et al., 2006; Fellman et al., 2010; Cory et al., 2011; Jollymore et al., 2012; Lee et al., 2015). Dissolved organic matter (DOM) is operationally defined as any organic material that is able to pass through a filter of a particularly identified pore size (e.g., typically 0.7 µm, 0.45 µm or smaller; Cory et al., 2011; Jollymore et al., 2012). Since DOM is typically about 50% C by mass (Cory et al., 2011), DOM is measured as DOC. DOC consists of a complex mixture of compounds that vary in size, weight, aromaticity, and reactivity, making it difficult to study DOC composition, and therefore its quality via direct molecular techniques. Hence, spectroscopy methods are a relatively quick and non-invasive means to observe and infer DOC and DOM composition and quality.

2.1 Absorbance Spectroscopy

UV-VIS absorbance spectroscopy is used to determine optical properties associated with the light-absorbing or chromophoric fraction of DOM (i.e. CDOM; Fellman et al., 2010; Cory et al., 2011). UV-VIS absorbance (i.e. absorbance from 220 to 720 nm) of CDOM has been shown to be a strong proxy (i.e. $r^2 > 0.95$, based on in-situ data) for DOC concentration (Waterloo et al., 2006; Jollymore et al., 2012; Jeong et al., 2012; Avagyan et al., 2014; Jones et al., 2014) and can also be used to infer aromaticity and molecular weight. It should be noted that UV-VIS absorbance may not be fully representative of all quality properties since CDOM only makes up a fraction of the total DOM pool (Cory et al., 2011).

Algorithms utilizing Beer Lambert's Law are used to determine DOC concentration from a given absorbance (e.g., 350 nm), as seen in Waterloo et al., 2006 (Equation 1).

$$DOC = 0.905\ x\ Absorbance_{350\ nm} \tag{1}$$

While specific UV absorbance at 254 nm (i.e. $SUVA_{254}$) is positively correlated with DOC aromaticity (Cory et al., 2011). Here, the absorbance at 254 nm is divided by the DOC concentration of the sample (units are L mg^{-1} C m^{-1}) (Equation 2). A wavelength of 254 nm is used because electron structures associated with aromatic C molecules absorb energy at this wavelength, while other structures do not (Weishaar et al., 2003). Therefore the absorbance reading is directly related to the amount of aromatic C within the total DOC sample.

$$SUVA_{254} = \frac{Absorbance_{254\ nm}}{DOC\ concentration} \tag{2}$$

And lastly spectral slope ratio (S_R) can be used to infer molecular weight. S_R is a dimensionless ratio of the slope of the 275 nm to 295 nm ($S_{275\text{-}295}$) absorbance spectra divided by the slope of the 350 nm to 400 nm ($S_{350\text{-}400}$) absorbance spectra (Equation 3) and exhibits a negative correlation with molecular weight.

$$S_R = \frac{S_{275\ nm\ -\ 295\ nm}}{S_{350\ nm\ -\ 400\ nm}} \tag{3}$$

2.2 Fluorescence Spectroscopy

In addition to chromophores, fluorophores (i.e. molecules that absorb and re-emit light) also make up a fraction of DOM in natural waters (Fellman et al., 2010). When a fluorophore (e.g., fluorescent DOM, FDOM) absorbs light energy, an electron is excited (i.e. transitions) from the original ground state to a higher electronic state (e.g., S0 to S2 in Figure 1). Within each electronic state there are numerous vibrational energy levels (e.g., 0, 1, 2 in Figure 1). The electron will often rapidly relax to the lowest vibrational energy level of S1 prior to emission, via internal conversion (Figure 1; Lakowicz, 2006). Then as the electron returns to ground state, the energy is emitted as light or fluorescence (Figure 1). The specific excitation and emission wavelengths at which fluorescence occurs is dependent on the concentration, chemical composition, and chemical structure (e.g., bonds between molecules) of the compound (Lakowicz, 2006; Fellman et al., 2010; Cory et al., 2011). Therefore, fluorescence can provide insight on DOC quantity and quality (e.g., redox state, reactivity, and source material; Fellman et al., 2010; Cory et al., 2011). However, it is important to note that FDOM only makes up approximately 1% of the total DOM pool (Cory et al., 2011). Therefore similar to CDOM, a limitation of using fluorescence spectroscopy is that only part of the "DOC pool" (rather than the "whole pool") is observable.

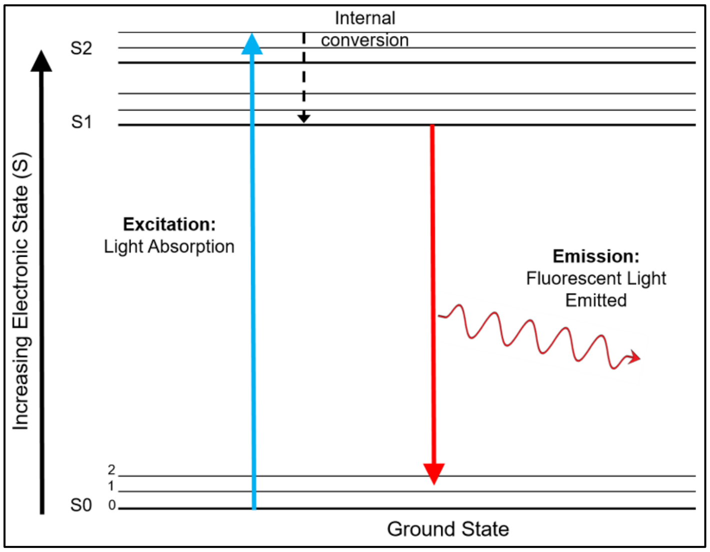


Figure 1. A simplified Jablonski diagram showing the process of fluorescence. Modified from Lakowicz (2006).

Despite its small fraction, FDOM is used as a proxy for total DOC concentration (Fellman et al., 2010; Cory et al., 2011). To do so, concentration of FDOM is measured in parts per billion (ppb) quinine sulfate equivalents (QSE), using a fluorometer, as seen in Saraceno et al. (2009), where V_{sig} is the output voltage for the sample, V_{CW} is the output voltage for clean (i.e. blank) water, and SF is an instrument specific scaling factor (Equation 4; Saraceno et al., 2009).

$$ppb\ QSE = \left(V_{sig} - V_{CW}\right) x\ SF \tag{4}$$

FDOM is also used to infer DOC quality properties. A fluorescence index (FI) value can be calculated to assess potential FDOM source material. The FI uses an excitation wavelength of 370 nm and the resulting emission intensities at 470 nm and 520 nm are then divided (Equation 5; McKnight et al., 2001; Fellman et al., 2010).

$$FI = \frac{470nm\ Emission\ Intensity}{520nm\ Emission\ Intensity} \tag{5}$$

High FI values (i.e. ~1.8) are indicative of microbial or autochthonous sources, while low FI values (i.e. ~1.2) are indicative of terrestrial or allochthonous sources (McKnight et al., 2001).

In addition to FI, three-dimensional excitation emission matrices (i.e. EEMs) are commonly used to assess DOC composition. EEMs are produced using multiple excitation wavelengths and measuring the resulting emission intensities across a range of wavelengths (Fellman et al., 2010; Cory et al., 2011). This technique is used frequently to study DOC composition in terms of molecular groupings and functions as EEMs contain a large amount of information on the origin and processing of DOC (Fellman et al., 2010; Cory et al., 2011). Additional optical properties including protein-like components (i.e. tyrosine-like and tryptophan-like components), humic-like components, the freshness index, humification index, and redox index can also be determined using fluorescence spectroscopy to better understand DOC composition in natural waters (e.g., see Fellman et al., 2010; Khamis et al., 2015; Beiroza and Heathwaite, 2016).

3. Technology and Limitations

3.1 In-Situ Optical Instruments

Previous work showed that DOC concentration and composition are dynamic and can change over short time scales (i.e. hours to days) (Carstea et al., 2009; Cory et al., 2011; Inamdar et al., 2011; Neal et al., 2012; Grayson and Holden, 2012; Strohmeier et al., 2013; Jones et al., 2014; Mast et al., 2016). Therefore, it necessary to collect long-term, high-frequency data when studying DOC export as small and/or rapid shifts in DOC quantity and quality are missed with less frequent sampling (Figure 2; Bowes et al., 2009; Inamdar et al., 2011; Jollymore et al., 2012; Jones et al., 2014; Blaen et al., 2016). In-situ optical measurements via long-term, field-deployable spectrophotometers and fluorometers enable researchers to collect this valuable high resolution temporal data using the spectroscopy methods discussed above. This review focuses on two, representative instruments frequently used in published field studies to date: 1) submersible FDOM fluorometers (e.g., WET Labs, WETStar FDOM fluorometer, Figure 3A) and 2) submersible spectrophotometers (e.g., s::can, spectro::lyser, Figure 3B). For brevity, this review specifically discusses the WET Labs FDOM sensor and the s::can spectro::lyser as well-documented examples of these technologies (i.e. these sensors have been and are used in many published studies). However, there are many other manufacturers developing and producing in-situ optical instruments based upon the same fundamental optical methods including, the SeaPoint UV fluorometer (SeaPoint Sensors, Inc.), the Cyclops-7 fluorometer (Turner Designs), the microFLU CDOM fluorometer (TriOS), the UviLux fluorometer (Chelsea Technologies Group), the Satlantic UV spectrophotometer, and the ABB AV400 UV absorbance probe.

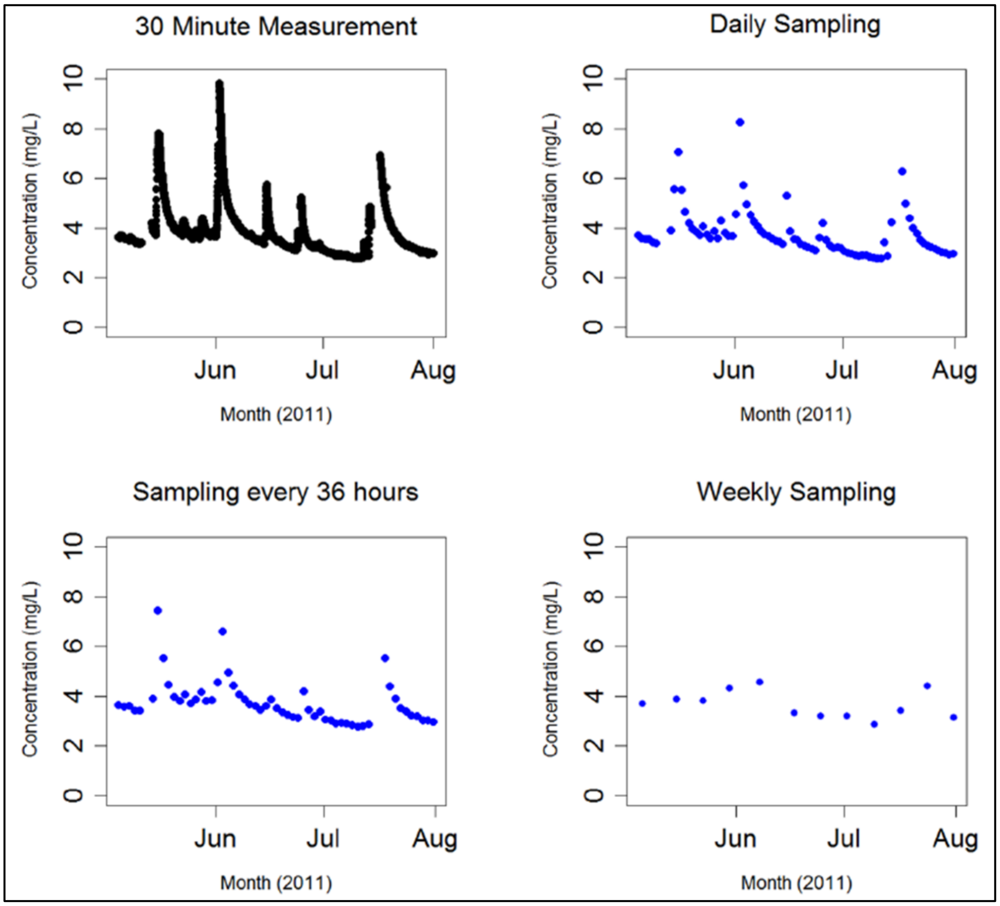


Figure 2. Plots of DOC concentration verses time created from data collected at different sampling intervals in order to highlight the importance of high-frequency sampling in DOC dynamics and export studies (Image source: Jollymore et al., 2012).

FDOM fluorometers use a light emitting diode (LED) as a light source and most have a fixed single excitation/emission pair; although there are less well documented and reliable scanning wavelength fluorometers, which measure the emission signal over a range of wavelengths (Saraceno et al., 2009; Lee et al., 2015). The WETStar FDOM fluorometer from WET Labs has a 7 mm optical path length along which light with a fixed wavelength of 370 nm is emitted to excite the FDOM in stream water. The resulting emission at 460 nm is measured to quantify the amount of FDOM present using Equation 4 (Saraceno et al., 2009). This measurement is typically taken at 1 hertz (Hz) for 30 seconds every 15 minutes, 30 minutes, or every hour and stored on a data logger (e.g., CR1000 data logger, Campbell Scientific). Most FDOM fluorometers can function at depths up to several hundred meters and across a wide temperature range of 0 to 30 degrees Celsius (°C; Bergamaschi et al., 2009). However, a temperature correction algorithm may need to be applied in studies with large temperature fluctuations (see Section 3.2). Many FDOM sensors also have automated systems to reduce biofouling (e.g., bio-wipers; Bergamaschi et al., 2009). FDOM sensors are relatively inexpensive (e.g., \$3000-6000) compared to CDOM sensors and have low power requirements (due to the use of LEDs), typically consuming 500 milliwatts or less (Bergamaschi et al., 2009; Conmy et al., 2014).

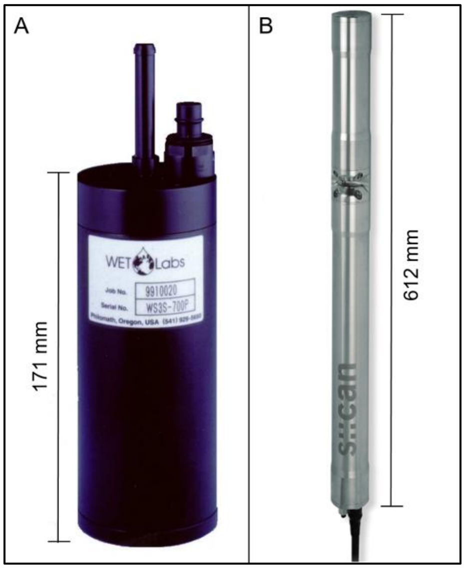


Figure 3. Images and dimensions of example in-situ optical sensors: A) WETStar FDOM Fluorometer from WET Labs and B) spectro::lyser from s::can. (Image from A) WET Labs and B) s::can).

On the other hand, submersible spectrophotometers measure absorbance across the UV-VIS spectrum. For example, the spectro::lyser from s::can was the first submersible multiparameter probe that could measure absorbance of CDOM in stream water over the UV-VIS range of 220 to 720 nm (Grayson and Holden, 2012). It can also measure NO_3-N, total suspended solids (TSS), and turbidity (Table 1). The spectro::lyser uses a xenon flash lamp and 256 pixel array detector to measure the resulting absorbance of CDOM at a 2.5 nm wavelength interval over an open path length (Jollymore et al., 2012). The path length on the spectro::lyser can be selected by user, providing users with several options to meet different water conditions. For example, an instrument with a path length of 100 mm can accurately measure DOC concentrations from approximately 0 to 15 mg/L (Jollymore et al., 2012; Grayson and Holden, 2016), however higher DOC concentrations (i.e. up to ~70 mg/L) can be measured using smaller path lengths of 35 mm or 5 mm, but with considerable reduced accuracy (Koehler et al., 2009; Grayson and Holden, 2016). Similar to FDOM sensors, these instruments also have systems to reduce biofouling. For example, they use bio-wipers or compressed gas to clean the optical window at a given interval of time (Etheridge et al., 2014a; Grayson and Holden, 2016). However, biweekly to monthly maintenance is still required as biofouling is not completely eliminated when using any antifouling system (Downing et al., 2009; Jollymore et al., 2012). The spectro::lyser can take measurements over the entire wavelength spectrum (i.e. 220 to 720 nm) at a rate as fast as every 2 minutes, but typical deployments monitor every 15 minutes, 30 minutes, or every hour, which are then stored on an internal data logger. External data loggers (e.g., CR1000 data logger, Campbell Scientific) can also be used with most of these in-situ sensors, including the spectro::lyser, for high-frequency sampling over extended deployment periods (e.g., monitoring at a 15 minute interval for greater than 2 weeks, which may exceed the internal memory). When compared to FDOM sensors, the spectro::lyser requires a larger power supply to power the xenon lamp, although there are numerous options for sustained power including battery, solar, and wind, which are helpful for remote field deployment.

3.2 Effects of Temperature on Sensors

It is important to note that temperature and turbidity can both interfere with optical measurements when using in-situ FDOM fluorometers and spectrophotometers. Temperature has an insignificant effect on absorbance readings of UV-VIS sensors, however signals received by FDOM sensors can be greatly affected due to thermal quenching (Baker, 2005; Downing et al., 2012; Conmy et al., 2014; Lee et al., 2015). As temperature increases it will cause electrons to return to the original ground state via radiationless decay, therefore reducing the fluorescence signal (i.e. reducing measured (raw) FDOM values by an average of 0.8-1.5% for every 1 °C from 1 to 25 °C) of FDOM (Watras et al., 2011; Downing et al., 2012; Lee et al., 2015). The extent of thermal quenching depends on the degree of exposure to the heat source, which varies with organic matter composition (Baker, 2005). In environments with large temperature fluctuations (e.g., >10 °C), thermal quenching can result in underestimating FDOM concentrations. Thus users of FDOM sensors should: 1) apply temperature compensation equations to field data (e.g., Watras et al., 2014) or 2) use in-situ spectrophotometers as an alternative in studies where temperature variability will be a factor (Lee et al., 2015).

3.3 Effects of Turbidity on Sensors

Turbidity is an inherent issue for both FDOM and UV-VIS sensors as it fundamentally interferes with light passage and detection. Suspended particles that make up turbidity can absorb and scatter light thus altering instrument sensor detection (Downing et al., 2012). As a result, UV-VIS sensor outputs increase linearly with increasing turbidity as the suspended particles absorb more light than the CDOM would alone. If not accounted for, this turbidity effect results in over estimating CDOM concentration. On the other hand for the FDOM sensor, the scattering of light due to suspended particles will reduce both the excitation signal from the FDOM light emitter and the resulting emission signal to the FDOM detector. If not accounted for, this turbidity effect results in underestimating FDOM concentration (Downing et al., 2012). In fact, FDOM sensors are more sensitive to turbidity than UV-VIS sensors, as FDOM signals decrease exponentially with increasing turbidity (Lee et al., 2015). Saraceno et al. (2009) demonstrated the effect of turbidity on FDOM measurements by including two FDOM sensors, one of which filtered the water prior to measurement and the other that left the water unfiltered. The study showed that at peak discharge during a storm flow event in a stream, when turbidity was highest, the unfiltered FDOM sensors received a dampened signal, thus underestimating the amount of FDOM in the stream by >10% during that point in time (Figure 4; Saraceno et al., 2009). Thus, FDOM field data should be calibrated using turbidity correction equations. To do so, an in-situ turbidimeter must be deployed to simultaneously measure turbidity (Downing et al., 2012). Once turbidity is known, correction equations can be developed based on the specific fluorometer and turbidimeter used as well as the matrix waters, since the extent of reduction of the FDOM signal due to light attenuation is dependent on both the instrument and nature (composition and size) of suspended particles (Downing et al., 2012; Khamis et al., 2015). For example equations see Saraceno et al. (2009), Downing et al. (2012), and Lee et al. (2015). To account for the turbidity effect on CDOM measurements, some UV-VIS instruments, including the s::can spectro::lyser, estimate turbidity directly from its UV-VIS measurements (Jollymore et al., 2012).

Due to the potential interference of temperature and turbidity, it is recommended that studies using in-situ optical instruments 1) use closed path FDOM sensors with a filtering system (e.g., see Saraceno et al., 2009) to reduce turbidity effects, and 2) deploy probes for temperature and turbidity in order to correct the FDOM signals received by the sensors with instrument dependent correction functions (Downing et al., 2012; Khamis et al., 2015; Lee et al., 2015). Similarly, turbidity measurements and corrections must be made for UV-VIS instruments, if turbidity correction equations are not already built into the sensor system.

In addition to specific temperature and turbidity interferences, there are a number of other more general logistical strengths and limitations to using FDOM and UV-VIS sensor systems in the field that have been discussed in other studies (e.g., Bergamaschi et al., 2009; Downing et al., 2012; Jollymore et al., 2012). We briefly summarize these strengths and limitations of the technology in Table 1.

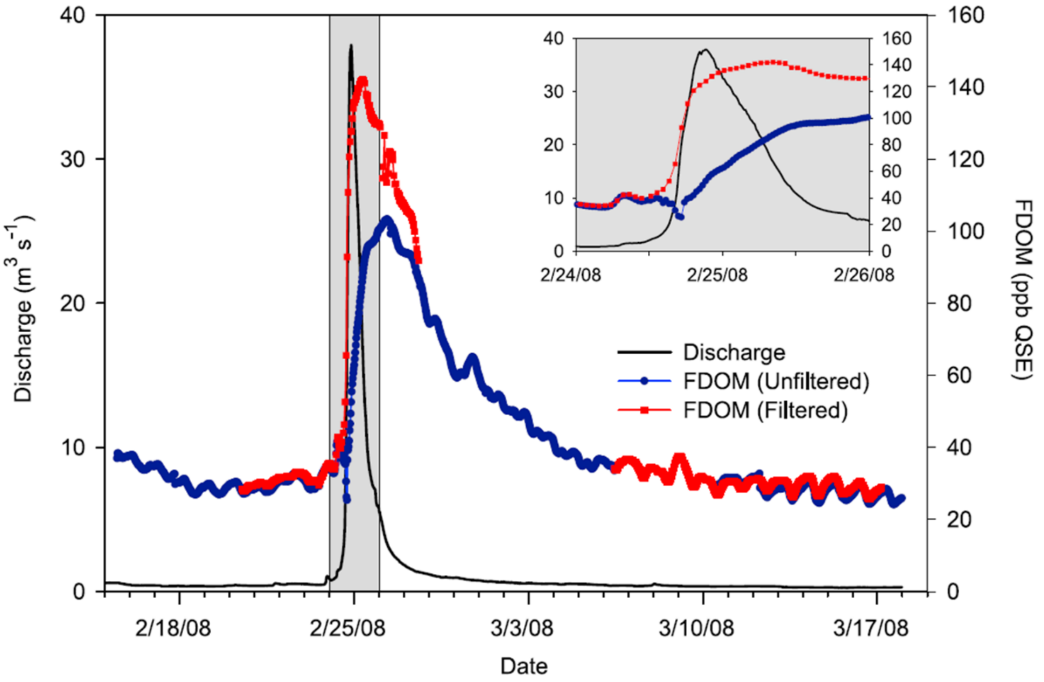


Figure 4. Plot of discharge and FDOM concentration verses time. Both unfiltered and filtered FDOM is plotted showing the importance of using a filter on in-situ FDOM sensors as turbidity can interfere with the sensor, reducing the FDOM signal (Image from Saraceno et al., 2009).

Table 1: Summary of the known strengths and limitations of in-situ optical instruments used to measure DOC in riverine systems.

Instrument Type	Strengths	Limitations
In-situ FDOM sensors	• Use in multiparameter probes • Functions at a range of depths; depth rating of 600 m • Relatively low power requirements; minimal need for sustained power supply • Communicates easily with most data loggers • Data collection frequency of minutes • Anti-biofouling devices available • Use in wide range of water types • Relatively inexpensive • No reagents needed, no waste products	• **Sensitive to only small fluorescent fraction of DOM** • Temperature interference • Turbidity interference • Biofouling still occurs and requires regular maintenance (biweekly to monthly) • General lack of user-support for system set-up and maintenance (e.g., interfacing between power supply and data logger) • Occasional data loss (e.g., due to power interruptions or exceeding internal memory)
In-situ UV-VIS sensors	• Use in multiparameter probes • Covers large range in DOC (typically 0-15 mg/L, but larger depending on path length) • Can also measure NO_3-N, TSS, Turbidity • Use in remote field areas • Compressed air and wipers to reduce biofouling • Little to no temperature interference • Data collection frequency of minutes • Numerous sustained power supply options (e.g., battery, wind, solar) • Use in wide range of water types • No reagents needed, no waste products	• **Sensitive to only small chromophoric fraction of DOM** • Turbidity interference • Biofouling still occurs and requires regular maintenance (biweekly to monthly) • General lack of user-support for system set-up and maintenance (e.g., interfacing between power supply and data logger) • Relatively large power demand and power supply infrastructure needed • Occasional data loss (e.g., due to concentrations outside of measurement range, power interruptions, or exceeding internal memory) • Expensive

4. Examples of Utility in Riverine DOC Studies

High-frequency, in-situ optical monitoring is emerging as a critical tool for understanding C transport and processing from hillslopes to streams and from streams to downgradient ecosystems (Waterloo et al, 2006; Saraceno et al., 2009; Jones et al., 2014; Blaen et al., 2016; Grayson and Holden, 2016; Raymond et al., 2016). In order to use in-situ optical methods (e.g., FDOM fluorometers and spectrophotometers) most effectively, they should be coupled with discrete DOC sampling (Downing et al., 2009; Saraceno et al., 2009; Pellerin et al., 2012; Sobczak and Raymond, 2015). The discrete samples allow for direct calibration of DOC concentrations measured from CDOM and FDOM proxies against laboratory measurements as well as allow for quantification of optical properties that require different measurements outside of the range of in-situ optical instruments (e.g., FI values). Automatic pump samplers (e.g., Teledyne ISCO automatic water sampler) are an option for remotely collecting these discrete DOC samples. Some recent studies focused on DOC cycling in stream catchments have leveraged these high-frequency, in-situ optical monitoring technologies to provide new insight on 1) seasonal, event, and diurnal variations in DOC quantity and quality*,* 2) sources and pathways of DOC to the stream channel and 3) annual DOC flux. These studies and insights are summarized below to illustrate potential future uses of these in-situ optical DOC sampling technologies.

4.1 Revealing Seasonal, Event, and Diurnal Variations in DOC Quantity and Quality

High-frequency sampling over extended time periods (e.g., multiple months to years) allows for analysis of changes in DOC across numerous time scales. Several studies with sampling periods of months to years have been able to observe seasonal variations in DOC quantity and quality consistent with other long-term DOC studies. For example, Wilson et al.

(2013) examined DOC processes in the steep, humid, forested catchment of Bigelow Brook in Massachusetts, USA. Using a Turner Designs Cyclops-7 CDOM fluorometer, Wilson et al. (2013) sampled at intervals of 15 minutes for approximately 300 days from October 2009 to November 2010. They observed that DOC concentrations were highest during the summer (July-August) and fall (September-October) seasons. A summer peak in DOC concentrations is also seen in many other regions. For example, Grayson and Holden (2012) saw the highest DOC concentrations during summer months in the upland peat catchment of Cottage Hill Sike in northern England and Jeong et al. (2012) observed large summer DOC concentrations in the mountainous catchment of Haean Basin in northern South Korea. Both Grayson and Holden (2012) and Jeong et al. (2012) were able to observe these seasonal trends using submersible spectrophotometers from s::can (Messtechnik GmbH, Austria). The consistency between results from DOC studies using in-situ optical methods (after site-specific calibration) and those from studies using low-frequency "grab samples," supports their ability to provide comparable measures of DOC in riverine systems and confirms our previous understanding of DOC dynamics across seasons. However, beyond just confirming these long-term seasonal patterns, they are revealing new shorter timescale patterns and processes (Figure 2).

Given the high sampling frequency of studies that incorporated in-situ optical instruments, these studies were also able to observe intra-seasonal and multiple individual event flow DOC dynamics, which was previously difficult to near impossible (Figure 2). For instance, Wilson et al. (2013) observed that the DOC response to storm events changed between seasons. DOC concentration increased rapidly with the onset of a storm flow during the summer and fall. DOC then quickly reached a peak concentration and remained elevated throughout the storm event, followed by a rapid decrease in concentration, tracking the decrease in discharge as the storm ceased. On the other hand, during the spring and winter seasons, DOC concentration showed slower rates of increase at the onset of an event, and DOC also reached a lower maximum concentration and declined more gradually after the event when compared to summer and fall events (Wilson et al., 2013). These seasonally consistent patterns were possible because the sensors captured all the storm events across all the seasons (Figure 2).

As another example of new insight, Saraceno et al. (2009) used in-situ optical methods (i.e. WETStar FDOM fluorometer coupled with discrete grab sampling) to examine DOC variations during individual storm events in the disturbed agricultural watershed of Willow Slough in Sacramento, California, USA. The fluorometer took measurements at 1 hour intervals for 4 weeks, during which there was one large precipitation induced storm flow event. Saraceno et al. (2009) observed that in-stream DOC concentration increased with discharge, in this case to 4 times the concentration observed during baseflow (Figure 4). By coupling the in-situ FDOM sensors with discrete sampling, Saraceno et al. (2009) was also able to measure DOC quality parameters such as $SUVA_{254}$, S_R, and FI (although $SUVA_{254}$ and S_R could also be measured had they used an in-situ UV-VIS sensor). These discrete samples paired with the FDOM data, indicated that the DOC in the stream channel during the event was largely of terrestrial origin illustrating that the storm DOC was primarily allochthonous and rapidly mobilized from hillslopes to the stream.

Additionally, in-situ optical instruments have been used to study DOC dynamics during snowmelt events. Pellerin et al. (2012) studied variations in DOC quantity and quality associated with a snowmelt event in the Sleeper's River watershed in Vermont, USA. Using a WETStar FDOM fluorometer, measurements were taken at 30 minute intervals for 2 months. Here, they also observed increasing DOC concentrations with increasing snowmelt. It should be noted that there are studies, which show clear decreasing or decoupled DOC concentration with discharge. Grayson and Holden (2012) examined DOC processes in upland peat catchment using an s::can spectro::lyser, which took measurements every 15 to 20 minutes from July to December 2009. They observed decreasing CDOM absorbance (i.e. CDOM concentration) along the rising limb of numerous storm hydrographs and overall found discharge and CDOM absorbance to be poorly correlated. Similarly, Koehler et al. (2009) used an s::can spectro::lyser to study DOC dynamics in an Atlantic blanket bog in south west Ireland. Sampling was conducted every 30 minutes over the course of 2007. DOC concentration was shown to have a weak negative correlation with discharge, and instead a strong correlation with temperature, indicating that temperature dynamics rather than flow dynamics are the primary control on DOC in these bog systems. These studies, where discharge and DOC concentration were inversely related, hypothesized that the decrease in DOC during the rising limb of the hydrograph is likely due to 1) dilution as the hydrological response in peatland catchments is different from steeper hillslope catchments and 2) that DOC production in the peatlands may be limited by the time in between storms (as microbes break down organic matter at times when the water table is low), and therefore the pool of DOC available for export is depleted over the wet season (Koehler et al., 2009; Grayson and Holden, 2012).

Being able to consistently monitor DOC responses across storm events and seasons will allow for new ways to compare watersheds and their responses to flow events. This is crucial to understand the annual DOC flux out of watersheds and delivered to downstream ecosystems and water resources, a large portion of which occurs during these intermittent flow events (Jones et al., 2014; Raymond et al., 2016). These in-situ optical sensors enable researchers to more safely and consistently sample during both low and high magnitude storm events, the timing of which is not always predictable (Figure 2).

At an even finer temporal resolution, in-situ optical sensors are revealing short-lived, diurnal changes in DOC quantity and quality (e.g., Spencer et al., 2007; Worrall et al., 2015) as a result of ecosystem processes (e.g., autochthonous DOC production). Spencer et al. (2007) used a WETStar FDOM fluorometer along with two in-situ spectrophotometers (AC-9 photometer for the visible range and a Satlantic UV spectrophotometer for the UV range) in order to assess diurnal variations in DOC in the San Joaquin River in California, USA. Spencer et al. (2007) observed that DOC does exhibit measurable diurnal variations. CDOM absorbance values peaked in the early evening and bottomed out at dawn, while CDOM fluorescence and S_R were highest in the early morning (Figure 5). In addition to the CDOM peak in the early morning, CDOM fluorescence exhibited a second peak at dusk. Through these unprecedented measurements, Spencer et al. (2007) were able to conclude that the decreasing daytime CDOM fluorescence is likely suggestive of photobleaching, while the imposed second CDOM peak on the overall diurnal trend is hypothesized to be the result of zooplankton grazing. When the S_R trends were compared to CDOM absorbance and fluorescence trends, the decreasing S_R values throughout the daytime help support the hypothesis that DOC quantity and quality is controlled on a diurnal scale by photochemical processing and in-situ biological production (possibly from zooplankton grazing). However, Spencer et al. (2007) acknowledges that the diurnal variations in DOC are complex and the controlling processes need to be further studied as these short-term DOC variations may complicate DOC source studies. These new hypotheses along with intra-seasonal, event, and diurnal DOC variations can now be more regularly evaluated across systems where these high-frequency measurements can be made. This will allow for cross-site, transferable understanding of how short-term processes may impact longer timescale observations and ecosystem processes.

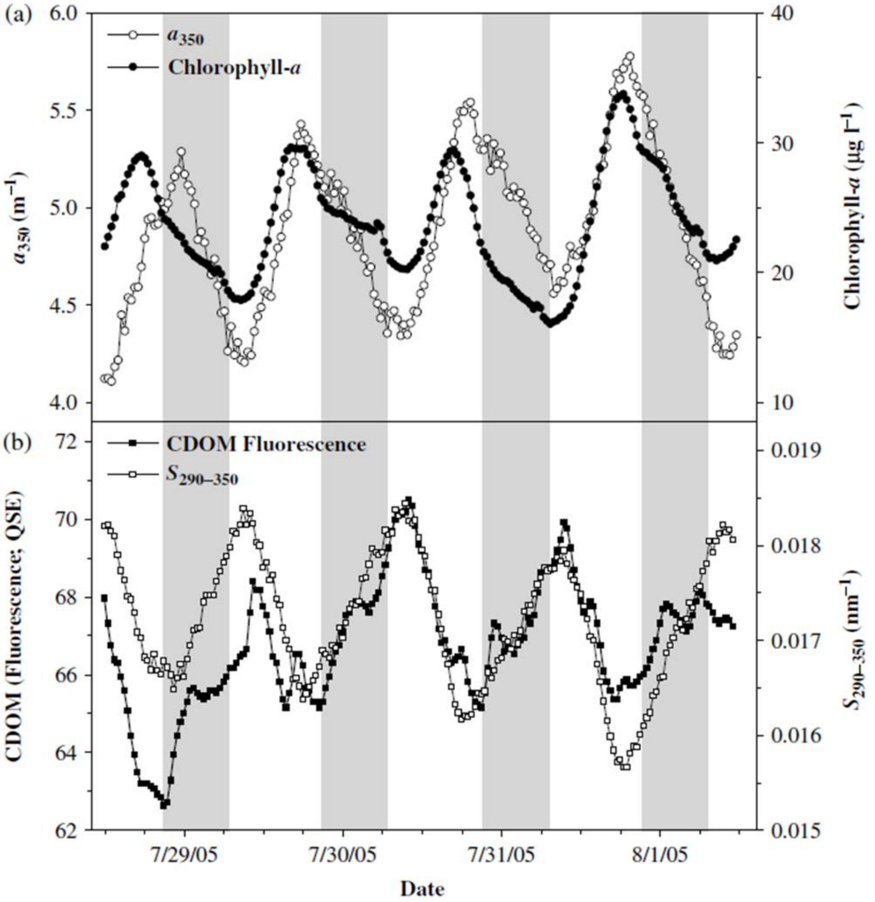


Figure 5. Diurnal trends for CDOM and absorbance. Absorbance peaks in the early evening, while CDOM peaks in the early morning (Image from Spencer et al., 2007).

4.2 Revealing Sources and Pathways of DOC to the Stream Channel

In-situ optical methods also provide insight on shifts in sources and pathways of DOC through time (e.g., during storm events, between seasons, etc.) by utilizing temporal data of DOC quantity and molecular characteristics. As mentioned earlier, Saraceno et al. (2009) observed that terrestrially-derived DOC increased in the stream channel during the storm event, by measuring

$SUVA_{254}$, S_R, and FI (again $SUVA_{254}$ and S_R could be measured using an in-situ UV-VIS sensor to achieve a higher sampling frequency). However, the FI values observed (i.e. 1.5-1.7) were higher than predicted for a disturbed agricultural watershed. Therefore, Saraceno et al. (2009) concluded that the terrestrially-derived DOC was likely "fresh" or less degraded, specifically coming from plant leachates in surface runoff over organic-rich agricultural soils. In addition, by conducting high-frequency sampling of FDOM using an in-situ optical sensor, Saraceno et al. (2009) observed a time lag between peak FDOM, discharge, and turbidity, where the FDOM lagged ~11 to 15 h behind peak discharge and turbidity. This suggests that in their disturbed agricultural watershed, shallow soil flowpaths remain important for several days after the precipitation event and that the suspended sediment (i.e. turbidity) and FDOM may have different watershed sources, because they peak at different times. Without the continuous high sampling frequency of in-situ optical sensors this lag in variables would likely have gone unnoticed.

A time lag between peak discharge and peak FDOM was also observed in other optically-based studies. Wilson et al. (2013) saw a similar length time lag between the FDOM and discharge during storm events in their forested headwater catchment as Saraceno et al. (2009), while Pellerin et al. (2012) observed a much shorter (~60 min) time lag during snowmelt events in Vermont, USA. The tight coupling of peak discharge and peak FDOM seen by Pellerin et al. (2012), along with their observed increasing $SUVA_{254}$ and decreasing FI values, indicated that surficial flowpaths along organic-rich soils were dominant during the snowmelt events (similar to Saraceno et al., 2009). Other studies also found that increasing DOC concentrations during storm events are likely derived from surface and shallow subsurface flowpaths. For example, Waterloo et al. (2006) used an s::can spectro::lyser to examine DOC variations in blackwater streams of the Amazon rainforest. Measurements were made at 30-minute intervals from February to September 2002. By additionally estimating shallow and deep groundwater flow using Darcy's Equation, Waterloo et al. (2006) was able to use the spectro::lyser data to determine that solute paths shifted from deeper groundwater regions to shallow subsurface layers in the valley soils that are rich in organic material during the many observed storm events.

Finally, high-frequency data can also be used to observe and characterize detailed hysteresis relationships between discharge and FDOM concentration to infer DOC sources and processing. Pellerin et al. (2012) observed a counter-clockwise hysteresis relationship (Figure 6), which is hypothesized to be the result of a delayed contribution from surface and shallow subsurface flowpaths and suggests that DOC export in the Sleeper's River watershed is transport limited rather than source limited. While many studies have also seen counter-clockwise hysteresis relationships for discharge and FDOM concentration (e.g., Strohmeier et al. 2013), some have observed clockwise hysteresis relationships. Jeong et al. (2012) saw a clockwise hysteresis relationship when studying DOC dynamics in the mountainous watershed in South Korea suggesting that DOC export in this watershed is source limited (i.e. DOC rich soil pools are depleted along the falling limb of the hydrograph). The discrepancy between hysteresis relationships for discharge and FDOM concentration is an important area of future research and may be dependent on watershed characteristics (e.g., slope, land cover, antecedent conditions, etc.). Having the ability to observe many hysteretic responses (via in-situ optical sensors) across a gradient of storm types in one watershed or across many watersheds will provide greater insight to what spatial and temporal watershed conditions are the primary controls on DOC concentrations and molecular characteristics. Overall, the ability to generate more transferable ideas and theories about DOC dynamics in watersheds and riverine systems is growing due to the ability of optical sensors to more consistently document DOC storm event dynamics across a huge range of watersheds (Figure 2).

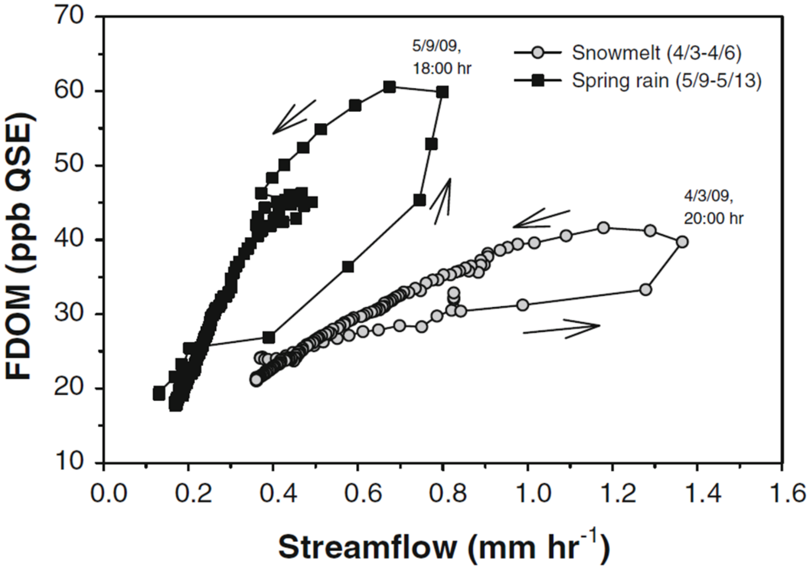


Figure 6. Counter-clockwise hysteresis observed during both snowmelt and precipitation events for FDOM (Image from Pellerin et al., 2012).

4.3 Annual DOC Flux

Previous work on sampling frequency, especially across dynamic stream flow conditions, found that infrequent sampling of DOC results in large underestimations of DOC fluxes (i.e. -10 to -88%), especially during moderate to low flow periods (Saraceno et al., 2009; Jollymore et al., 2012; Pellerin et al., 2012; Wilson et al., 2013; Jones et al., 2014; Grayson and Holden, 2016). As a result high-frequency, in-situ sampling of FDOM concentration should be used to improved DOC flux quantification. Thus far, studies that have incorporated in-situ optical sensor methods were able to better calculate short-term and annual DOC fluxes from watersheds (e.g., Koehler et al., 2009; Jones et al., 2014).

Most studies using in-situ optical sensors found that a significant amount of the annual DOC flux is exported during isolated brief storm events or, if in a more Mediterranean climate, during the wet season. Wilson et al. (2013) found that 63% of the annual DOC flux was exported during these brief, reoccurring hydrologic storm events, while Koehler et al. (2009) and Jeong et al. (2012) found that 45% and 48% respectively, was exported during storm events. Additionally, Waterloo et al. (2006) observed that 69 to 72% of annual export occurred during the wet season, all of which corroborates the need to sample on intra-seasonal and diurnal time scales. Waterloo et al. (2006) also quantified a relationship between DOC flux and stream discharge. The largest fluxes of DOC were typically seen in the top 10% of high flows (Koehler et al., 2009), thus as stream discharge increases, DOC flux will typically increase and in a predictable manner. For example, given the high temporal characterization of DOC across a large range of flow conditions (Figure 7), Waterloo et al. (2006) was able to generate two empirical models for DOC flux as there appears to be a threshold behavior in the specific DOC flux response to a given discharge (Figure 7). Specifically Waterloo et al. (2006) showed that DOC flux, F_{DOC}, when stream flow (Q_{stream}) is less than 2.8 mm d^{-1} (Equation 6; $r^2 = 0.79$) is,

$$F_{DOC} = 0.0075\ x\ Q_{stream}{}^{1.5630} \tag{6}$$

and when Q_{stream} is greater than 2.8 mm d^{-1} (Equation 7; $r^2 = 0.99$).

$$F_{DOC} = 0.0278\ x\ Q_{Stream} - 0.0432 \tag{7}$$

Overall, more accurate estimates of flux dynamics were possible using the high resolution in-situ optical sensors in both cases of flux budgets and flux response to changes in flow.

Understanding these consistently observed “pulses” of DOC in response to variable flow conditions is important to future research of ecosystem processes at the watershed scale.

Specifically, C gains and losses are important for understanding and accurately quantifying net ecosystem productivity and net ecosystem C balance (Wilson et al., 2013). Thus, missing or misappropriating the key portion of C in the form of DOC will result in erroneous net ecosystem C balance models. In addition, understanding changes in sources and pathways of DOC to the stream channel is key to determining DOC quality moving through riverine environments, because DOC quality is largely dependent on C source material (Cory et al., 2011). The timing of both DOC quantity and molecular characteristics impacts DOC bioavailability and, therefore, timing of DOC flux can have a large effect on organisms, downstream ecosystems, and water quality. These in-situ sensors seem to be capable of greatly improving our ability to "take the pulse" of key ecosystem parameters, specifically, DOC moving through watersheds and ecosystems (Roley et al., 2014). In taking the pulse of DOC in streams, we are also able to use that information to guide the study design and monitoring of other watershed attributes that control ecosystems and water quality, such as limiting nutrients (Blaen et al., 2016).

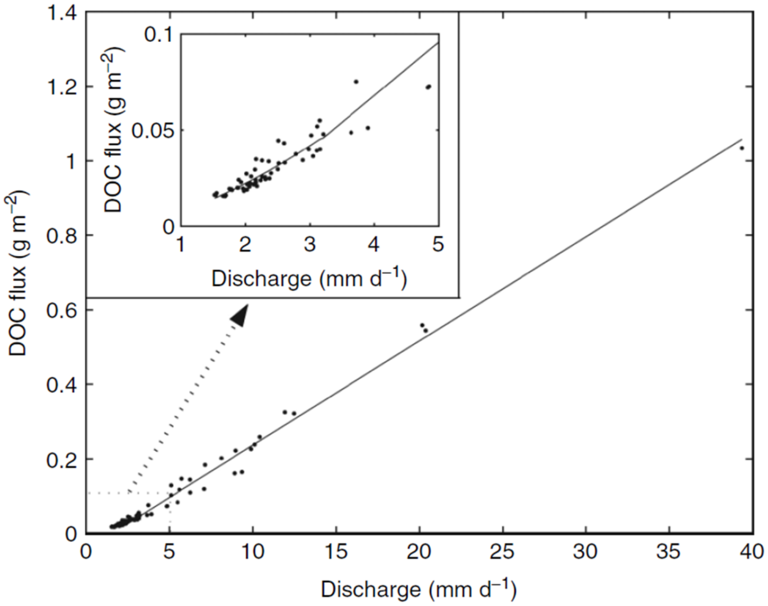


Figure 7. DOC flux increases linearly with discharge, although the relationship shifts slightly from low to high discharge (Image from Waterloo et al., 2006).

5. Conclusions & Future Considerations

High-frequency, in-situ optical monitoring allows for much higher resolution temporal observations of DOC processes in riverine environments than ever previously thought possible (i.e. dynamics operating on temporal scales of seconds to minutes, Figure 2 and 5). Thus, new processes and understanding of DOC in watershed and riverine systems are being revealed. In particular, these sensors are facilitating novel observations, conceptual understanding, and models that address fundamental questions about watershed DOC quantity and quality through time, the sources and pathways of DOC to the river network, and DOC mass flux. Future research on these important DOC dynamics will benefit from the use of these technologies. These early adopters of these in-situ optical sensor technologies have already made great advances as discussed above.

The in-situ optical methods are quick, precise, and relatively inexpensive when compared to collecting discrete water samples in the field and later completing chemical C analyses. However, they still have limitations that must be addressed by users. In particular, in-situ FDOM fluorometers and spectrophotometers should still be coupled with some discrete sampling to include the full range of DOC optical properties as well as cross check DOC concentrations against CDOM and FDOM proxies. In addition, temperature and turbidity correction equations should be applied (if not already built-in within the sensor system), especially in watersheds with large temperature fluctuations and/or high suspended sediment loads. There are emerging methods to make these temperature and turbidity corrections as this is an active area of research. It is worth noting again, as discussed in Section 2.1 and 2.2, FDOM and CDOM sensors only measure a fraction of the total DOM pool so all interpretation of bulk DOM processes and dynamics must be done in light of this limitation. Nonetheless, these in-situ optical methods can provide valuable information on DOC processes in streams and watersheds that is otherwise logistically prohibitive and therefore unavailable. As more users adopt this technology, user-support will also increase, eliminating numerous potential concerns (e.g., interfacing with power supplies and data loggers). In addition, these sensors could be used to standardize protocol between research groups and environmental regulators, creating opportunity for better networking and data sharing plans. As the climate and anthropogenic uses of land and water continues to change, in-situ optical instruments are likely to become crucial to measuring the pulse of riverine and watershed responses to these changes. Having this information will help to diagnose and understand both associated short- and long-term changes in DOC quantity and quality, C cycling in watersheds, as well as help guide the development of future watershed-scale management strategies.

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