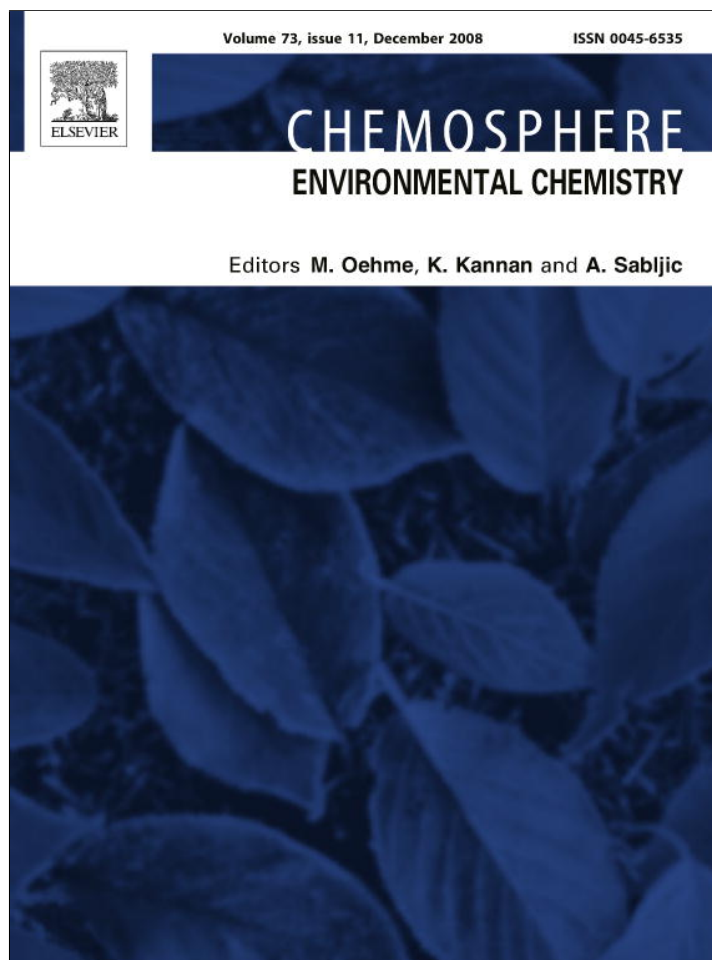


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## Relating dissolved organic matter fluorescence and functional properties

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### ABSTRACT

The fluorescence excitation–emission matrix properties of 25 dissolved organic matter samples from three rivers and one lake are analysed. All sites are sampled in duplicate, and the 25 samples include ten taken from the lake site, and nine from one of the rivers, to cover variations in dissolved organic matter composition due to season and river flow. Fluorescence properties are compared to the functional properties of the dissolved organic matter; the functional assays provide quantitative information on photochemical fading, buffering capacity, copper binding, benzo[*a*]pyrene binding, hydrophilicity and adsorption to alumina. Optical (absorbance and fluorescence) characterization of the dissolved organic matter samples demonstrates that (1) peak C (excitation 300–350 nm; emission 400–460 nm) fluorescence emission wavelength; (2) the ratio of peak T (excitation 220–235 nm; emission 330–370 nm) to peak C fluorescence intensity; and (3) the peak C fluorescence intensity: absorbance at 340 nm ratio have strong correlations with many of the functional assays. Strongest correlations are with benzo[*a*]pyrene binding, alumina adsorption, hydrophilicity and buffering capacity, and in many cases linear regression equations with a correlation coefficient >0.8 are obtained. These optical properties are independent of freshwater dissolved organic carbon concentration (for concentrations <10 mg L<sup>-1</sup>) and therefore hold the potential for laboratory, field and on-line monitoring and prediction of organic matter functional properties.

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### 1. Introduction

Dissolved organic matter (DOM) has a number of ecological and geochemical functions, including light absorption, proton binding, binding of heavy metals, aluminum and radionuclides, binding of organic contaminants, adsorption at surfaces, aggregation and photochemical reactivity (Perdue and Gjessing, 1990; Kullberg et al., 1993; Hessen and Tranvik, 1998). Information about these functional properties has been obtained largely from laboratory experiments with isolated fractions, especially humic and fulvic acids: exceptions include the 'NOM Typing project' (Gjessing et al., 1999), and Thacker et al. (2005, in press) and Gondar et al. (2008). In the latter studies, the authors describe a set of functional assays for DOM. These are simple, reproducible measurements that provide information about the environmental roles of DOM rather than its more basic physico-chemical properties. A key aspect of their approach was the use of a quality control standard, Suwannee River Fulvic Acid (SRFA) that was repeatedly put through the suite of assays in order to characterize their reproducibility. Another was the application of the assays to samples contrasting surface waters, one lake and three streams, in order to explore both temporal and

spatial variability in the functional properties of freshwater DOM. Thacker et al. (2005) showed that variability in the assay results for the lake and river DOM samples was statistically and significantly greater than that of the SRFA standard for eight of the eleven assays; the three functional properties that did not vary among the DOM samples were photochemical fading, copper binding and benzo[*a*]pyrene binding. Gondar et al. (2008) investigated seasonal variability in assays for the lake waters site. Seasonal trends were observed in six of the assays, which were explained by a simple mixing model in which the two end-members were DOM from the catchment (allochthonous) and DOM produced within the lake (autochthonous). The fraction of autochthonous DOM predicted by the model was significantly correlated ( $p < 0.01$ ) with chlorophyll concentration, consistent with production from phytoplankton. Autochthonous DOM was shown to be less light-absorbing, less fluorescent, more hydrophilic, and to possess fewer proton-dissociating groups than allochthonous material.

Thacker et al. (2005, in press) and Gondar et al. (2008) undertook functional assay analysis of DOM samples that included the measurement of DOM fluorescence properties. Fluorescence excitation–emission matrices (EEMs) have now become widely used to characterize and source DOM (see review by Hudson et al., 2007). Recent applications include the characterization of algal derived DOM (Nguyen et al., 2005); characterizing DOM produced

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by larvaceans and copopods (Urban-Rich et al., 2006); and the characterization of water extractable DOM in soil profiles (Corvace et al., 2006). However, Thacker et al. (2005, in press) and Gondar et al. (2008) analysed just one fluorescence parameter as part of their characterization of functional variability; that of the fluorescence intensity at just one excitation wavelength (340 nm) normalized to DOC. Much greater information is contained within a fluorescence EEM, which has been shown to relate to DOM character. For example, previous studies have indicated that the wavelength of fluorescence emission for fluorescence excited at ~300–340 nm (often called 'fulvic-like' fluorescence or fluorescence 'peak C') may relate to DOM aromaticity or hydrophobicity (Kalbitz et al., 1999). The intensity of fluorescence emitted at 330–370 nm after excitation at either 220–235 nm or 270–280 nm ('tryptophan-like fluorescence' or peak 'T') has been demonstrated to relate to both algal and microbial derived OM (Nguyen et al., 2005; Hudson et al., 2008). 'Peak C' fluorescence intensity normalized to absorbance at 340 nm has been demonstrated to relate to DOM molecular weight (Stewart and Wetzel, 1980).

In this study, we now analyse the fluorescence EEMs for the exact same samples used to assess the functional characteristics of DOM reported by Thacker et al. (2005, in press) and Gondar et al. (2008). We hypothesize that, by using the greater amount of available data in an EEM, compared to the simple fluorescence intensity measurement used in previous studies, we will identify additional correlations between DOM fluorescence and their function properties. To do this, we use the EEM fluorescence properties firstly to characterize the DOM samples, and then to derive relationships between this fluorescence derived DOM character and DOM function. In particular, we focus on the identification of optical properties that are potentially independent of freshwater DOM concentration over the range of DOM concentrations expected in natural and treated waters. For example, (1) the emission wavelength of 'maximum fulvic-like' fluorescence intensity; and (2) ratios of the intensity of maximum peak fluorescence, for example the ratio of 'fulvic-like' and 'tryptophan-like' fluorescence. These ultimately have the greatest potential to provide concentration independent measurements of DOM character and function.

## 2. Materials and methods

### 2.1. DOM samples

Samples are the exactly same samples detailed in Thacker et al. (2005, in press) and Gondar et al. (2008). Brief site details are provided again here. Samples were taken from four sites. Esthwaite Hall Beck (samples EHB1 and EHB2; 54°21'N, 2°59'W) is a stream draining a catchment of area ca. 1 km<sup>2</sup> comprising brown earth soils overlying Silurian slates (Palaeozoic slaty mudstone and siltstone). The catchment land cover is mainly mixed woodland, with some pasture. Esthwaite Water (samples EW1–10; 54°21'N, 2°59'W) is a eutrophic lake of catchment area 17.1 km<sup>2</sup>, surface area 1.00 km<sup>2</sup> and mean depth 6.4 m (Ramsbottom, 1976). The lake thermally stratifies in summer, and then has an anoxic hypolimnion. Samples were taken from the outflow or shore and represent epilimnetic water. The catchment soils consist of brown earths, cambic stagnohumic gleys and brown podzols, overlying Silurian slates (Palaeozoic slaty mudstone and siltstone). The catchment land cover is mixed woodland and pasture. The catchment includes EHB (see above). Two samples were replicated (EW4 and EW10). Gais Gill (samples GG1 and GG2, 54°24'N, 2°26'W) is a stream draining ferric stagnopodzols overlying Palaeozoic slaty mudstone and siltstone. The catchment area is moorland with an area of ca. 1 km<sup>2</sup>. Rough Sike (samples RS1–RS9; 54°41'N, 2°22'W) is a stream draining blanket peat of total depth 1–4 m, which has accumulated

on glacial clay till overlying Carboniferous limestone, sandstone and shale (Heal and Smith, 1978). The catchment has an area of ca. 1 km<sup>2</sup>. The vegetation is principally Eriophorum-Calluna and Sphagnum.

To investigate DOM functional variability through time, multiple samples were taken from two of the sites. These additional samples were taken at approximately monthly samples from Esthwaite Water from January 2005 (EW3) to September 2005 (EW10), to capture seasonal variability in lake productivity (Gondar et al., 2008). At Rough Sike, samples were collected at a wide range of flow regimes, sampled on 27/08/03, 02/06/04, 26/01/05, 16/03/05, 11/01/06, 21/05/06, 05/07/06, 11/10/06 and 15/11/06 (Thacker et al., in press).

In all cases samples (20–50 L) were collected in thoroughly rinsed 10 L plastic containers, that had been used numerous times previously for water collection; therefore "bleeding" of DOM would have been minimal. On return to the laboratory, the sample was filtered through Whatman GF/F filters (nominal pore size 0.7 µm), and then stored in the dark at 4 °C. Sample concentration was then performed to provide a DOM solution that could be subsequently diluted to a constant DOC concentration for use in the assays. In brief, this involved concentration to approximately 500 cm<sup>3</sup> by rotary evaporation operating with a water bath temperature of 45 °C, and a vacuum of 10 mbar. The concentrate was passed through Amberlite IR-120 resin, filtered sequentially through Whatman GF/F and Millipore 0.22 µm filters. The final isolate was stored at 4 °C in the dark. They comprised solutions of DOM (148–599 mg L<sup>-1</sup> DOC) in an electrolyte medium consisting of Na<sup>+</sup>, together with strong acid anions (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>) and HCO<sub>3</sub><sup>-</sup>. Further details can be found in Thacker et al. (2005).

SRFA (International Humic Substances Society) was used as a quality control standard. A stock solution of SRFA was prepared by adding 0.0445 g of solid SRFA to 200 cm<sup>3</sup> of ultra-pure water.

### 2.2. Laboratory methods

For both assay and fluorescence analysis, solutions containing 10 mg L<sup>-1</sup> DOC were prepared in duplicate, in a background of 0.1 M NaCl and 0.001 M phosphate buffer (pH 7). Fluorescence spectra were only determined for the 10 mg L<sup>-1</sup> solutions and not the raw water, so no information is available to determine whether significant changes in fluorescence properties occurred during the sample concentration stage. Fluorescence was measured in 4 cm<sup>3</sup> capacity (1 cm<sup>3</sup> path length) cuvettes using a Varian Cary Eclipse fluorescence spectrophotometer, equipped with a multicell holder with Peltier temperature controller enabling the measurement of excitation-emission matrices (EEM) at 20.0 ± 0.1 °C. To generate an EEM, excitation wavelengths were scanned from 200 to 400 nm in 5 nm steps, and the emitted fluorescence detected between 280 and 500 nm in 2 nm steps. Excitation and emission slit widths were 5 nm. Scan speed was 9600 nm min<sup>-1</sup>, permitting collection of a complete EEM in ~60 s. A subset of samples were analysed in triplicate to obtain a measure of instrument variability, and a further subset were analysed as a dilution series in order to confirm a linear relationship between fluorescence intensity and concentration. Manufacturer generated corrections for excitation and emission were used to correct the sample EEMs. To standardise the fluorescence intensity measured on different days, we also measured the strength of the Raman signal of deionised water in a sealed cuvette at excitation 348 nm (emitted between 395 and 400 nm) and all results are standardised to a mean Raman peak of 20 intensity units. Our results can be compared to a Quinine Sulphate standard; 32.5 intensity units are equivalent to 1 Quinine Sulphate Unit (1 µg L<sup>-1</sup> in 0.1 M H<sub>2</sub>SO<sub>4</sub>).

**Table 1**  
Summary of functional assays

Assay	Assay result	Assay number	Abbreviation
Photochemical fading	% loss in DOM absorbance at 340 nm	4	A <sub>340</sub> loss%
Buffering capacity	Acid groups titrated between pH 4 and 8 (meq g C <sup>-1</sup> )	5	AC <sub>4-8</sub>
Copper binding	Conditional stability constant (L g C <sup>-1</sup> )	6	logK <sub>c</sub>
Benzo[a]pyrene binding	Partition coefficient (cm <sup>3</sup> g C <sup>-1</sup> )	7	logK <sub>p</sub>
Hydrophilicity (DOC)	% of DOC not adsorbed DAX-8 resin at pH 2	8	Hyphil <sub>DOC</sub> %
Hydrophilicity (absorbance)	% of DOM absorbance (340 nm) not adsorbed by XAD-8 or DAX-8 resin at pH 2	9	Hyphil <sub>A340</sub> %
Alumina adsorption (DOC)	% of DOC adsorbed at pH 4	10	Ads <sub>DOC</sub> %
Alumina adsorption (absorbance)	% of DOM absorbance (340 nm) adsorbed at pH 4	11	Ads <sub>A340</sub> %

See Thacker et al. (2005) and Gondar et al. (2008) for further details.

Absorbance measurements and functional assays were performed as detailed in Thacker et al. (2005). Assays reported here provide quantitative information on photochemical fading, buffering capacity, copper binding, benzo[a]pyrene binding, hydrophilicity and adsorption to alumina. The assays are summarised in Table 1 and detailed experimental methodologies can be found in Thacker et al. (2005). Statistical analyses were performed using SPSS(c).

### 3. Results and discussion

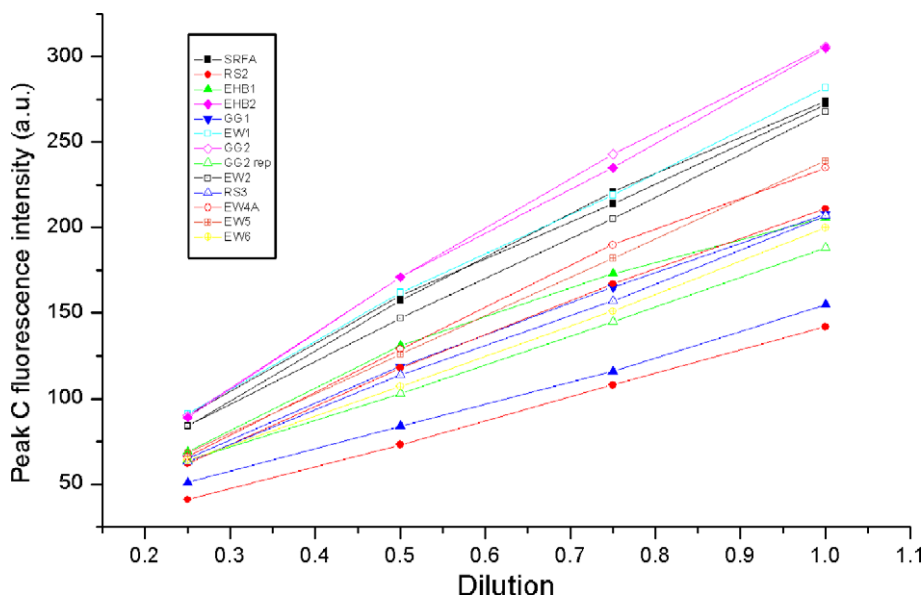
For all EEMs, three fluorescence peaks were identified that were always detectable. These were (1) fluorescence excited between 300 and 340 nm excitation, and emitted between 400 and 460 nm (peak C), (2) fluorescence excited between 220 and 250 nm excitation, and emitted between 400 and 460 nm (peak A), and (3) fluorescence excited between 220 and 235 nm and emitted between 330 and 370 nm (peak T). Both peak C and peak A fluorescence are attributed to fulvic-like and humic-like substances, and peak T to microbially and allogenically derived tryptophan-like substances; for a review, see Hudson et al. (2007). The second peak T fluorescence excited at 270–280 nm was not recorded, as significant background fluorescence from the more intense peak C fluorescence occurred at this location. For each peak, the excitation and emission wavelengths and the intensity of emitted fluorescence were recorded. Supplementary Table 1 presents a summary of both fluorescence and absorbance analyses and functional assays on the DOM samples. Additionally, mean and standard deviation of the fluorescence analyses of the SRFA standard analysed at 20 mg L<sup>-1</sup> concentration are tabulated. All results

are mean of the duplicate analyses in the case of DOM samples, and the mean of 30 duplicate samples in the case of SRFA standards.

#### 3.1. Dilution series and replicate analyses

Sixteen samples, four SRFA standards and twelve samples representing the four sample sites, were analysed as a dilution series to confirm that reabsorption effects (the so-called 'inner filter effect') did not affect the analyses (Mobed et al., 1996; Ohno, 2002). Samples were run undiluted and at 3:1, 2:2 and 1:3 dilutions. Results are presented in Fig. 1 for peak C, and show that fluorescence intensity of this peak decreases linearly with dilution. Linear regression of fluorescence intensity against concentration yielded  $R^2$  values of >0.99 for all samples except EHB1 ( $R^2 = 0.97$ ). Therefore, at these concentrations (<20 mg L<sup>-1</sup>) and absorption coefficients (<0.4 cm<sup>-1</sup> at 340 nm), fluorescence intensity of peak C has a linear relationship with concentration. Peak A showed a similar relationship; peak T intensity was generally too low for reliable diluted analyses.

Eight samples were analysed as instrument triplicates (data not shown). Triplicate analyses reproduced peak A excitation wavelength with a 1 $\sigma$  error of 1 nm, and emission wavelength of 3 nm error. Peak A intensity had a mean 1 $\sigma$  error of 15 intensity units, corresponding to a ~2–5% uncertainty in intensity measurements. Peak C excitation wavelength was reproduced with a 3 nm 1 $\sigma$  uncertainty and emission wavelength with a 4 nm uncertainty. Fluorescence C intensity reproduced with a mean 1 $\sigma$  error of 6 intensity units, corresponding to a 1–5% uncertainty in intensity measurements. Peak T intensity was reproduced with a mean 1 $\sigma$



**Fig. 1.** Fluorescence peak C intensity dilution series for DOM and SRFA samples.

error of 3 intensity units, corresponding to an uncertainty of <10% in intensity measurements. Sample preparation duplicates had similar errors to the instrument triplicates, suggesting that any difference between laboratory duplicates was much less than instrumental error. These results are similar to those reported in Thacker et al. (2005), where repeat analyses were made in two cases and replication was found to be within 3%.

3.2. Fluorescence characterization of DOM samples

Fig. 2a–c presents the fluorescence and absorbance properties of the DOM samples, together with the mean value of the SRFA 20 mg L<sup>-1</sup> concentration standard. Fig. 2a plots peak C fluorescence intensity against peak T fluorescence intensity, remembering that

all freshwater samples are at a constant concentration of 10 mg L<sup>-1</sup>. Esthwaite Water samples are characterized by higher peak T fluorescence intensities than most of the river samples. Peak T fluorescence is associated with microbial activity (Cammack et al., 2004; Elliott et al., 2006), increased algal production (Nguyen et al., 2005) and DOM breakdown by grazers (Urban-Rich et al., 2006), and because of this is also an indicator of anthropogenic pollutant DOM (Baker, 2001; Hudson et al., 2007; Hudson et al., 2008). Due to the fluorescence efficiency of the organic matter that comprises peak T, previous studies have demonstrated no correlation between peak T intensity and DOC for natural river systems. Samples in Fig. 2a with high peak T fluorescence intensity per g C would therefore suggest a greater concentration of this DOM fraction, although this still comprises a very small part of the total

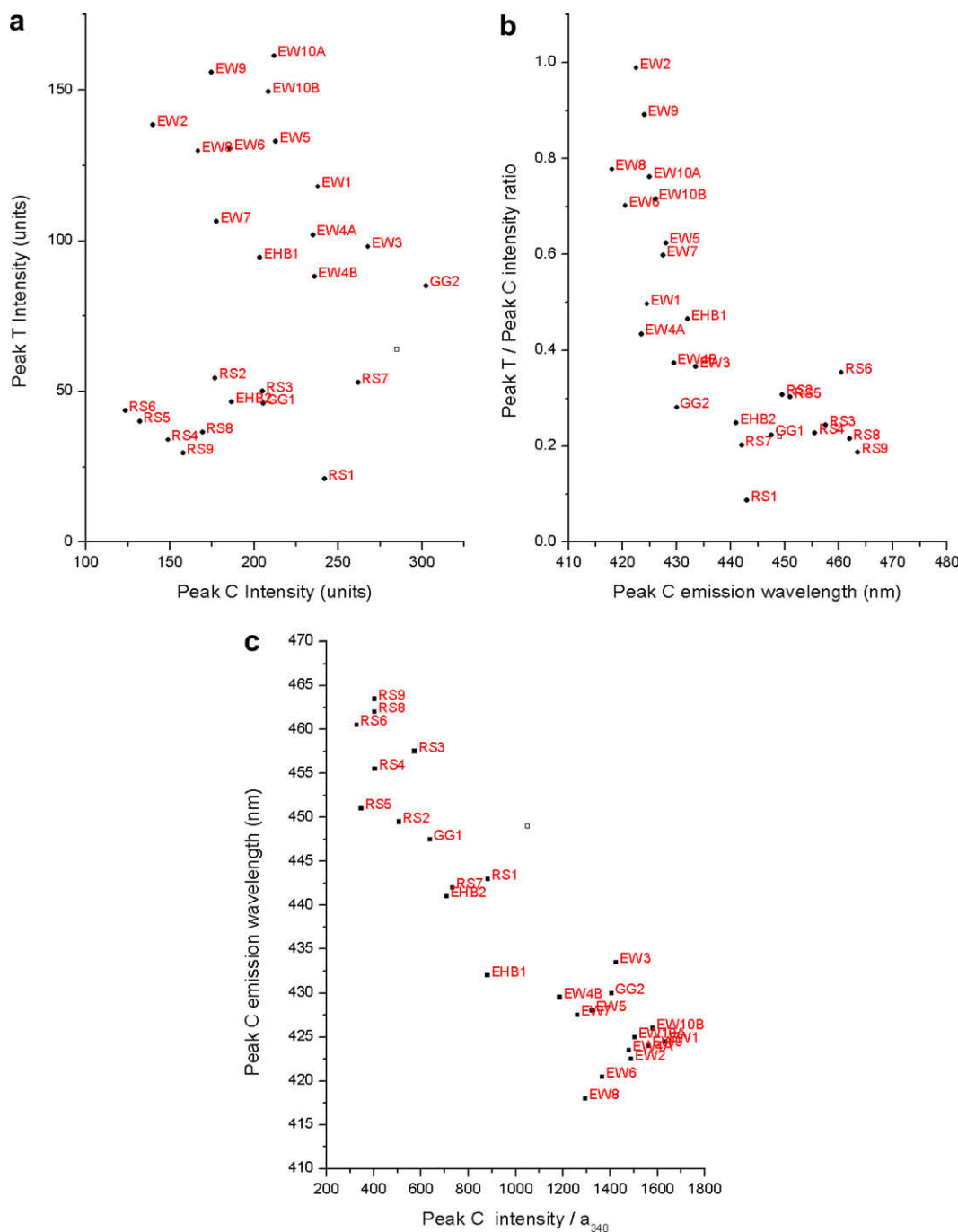


Fig. 2. Relationships between fluorescence parameters. (a) Fluorescence peak C intensity vs. fluorescence peak T intensity, (b) fluorescence peak C emission wavelength vs. peak T/peak C intensity ratio, (c) fluorescence:absorbance ratio vs. peak C emission wavelength. Mean value of the IHSS SRFA standard is shown by the open square.

DOC. Peak C fluorescence intensity, in contrast, has a strong correlation with DOC concentration in natural river systems. With our samples normalized to a constant DOC concentration, an increase in peak C intensity per g C most likely indicates an increased proportion of fluorescent fulvic-like and humic-like material in the total DOM load. Peak C intensity does not discriminate between sample sites.

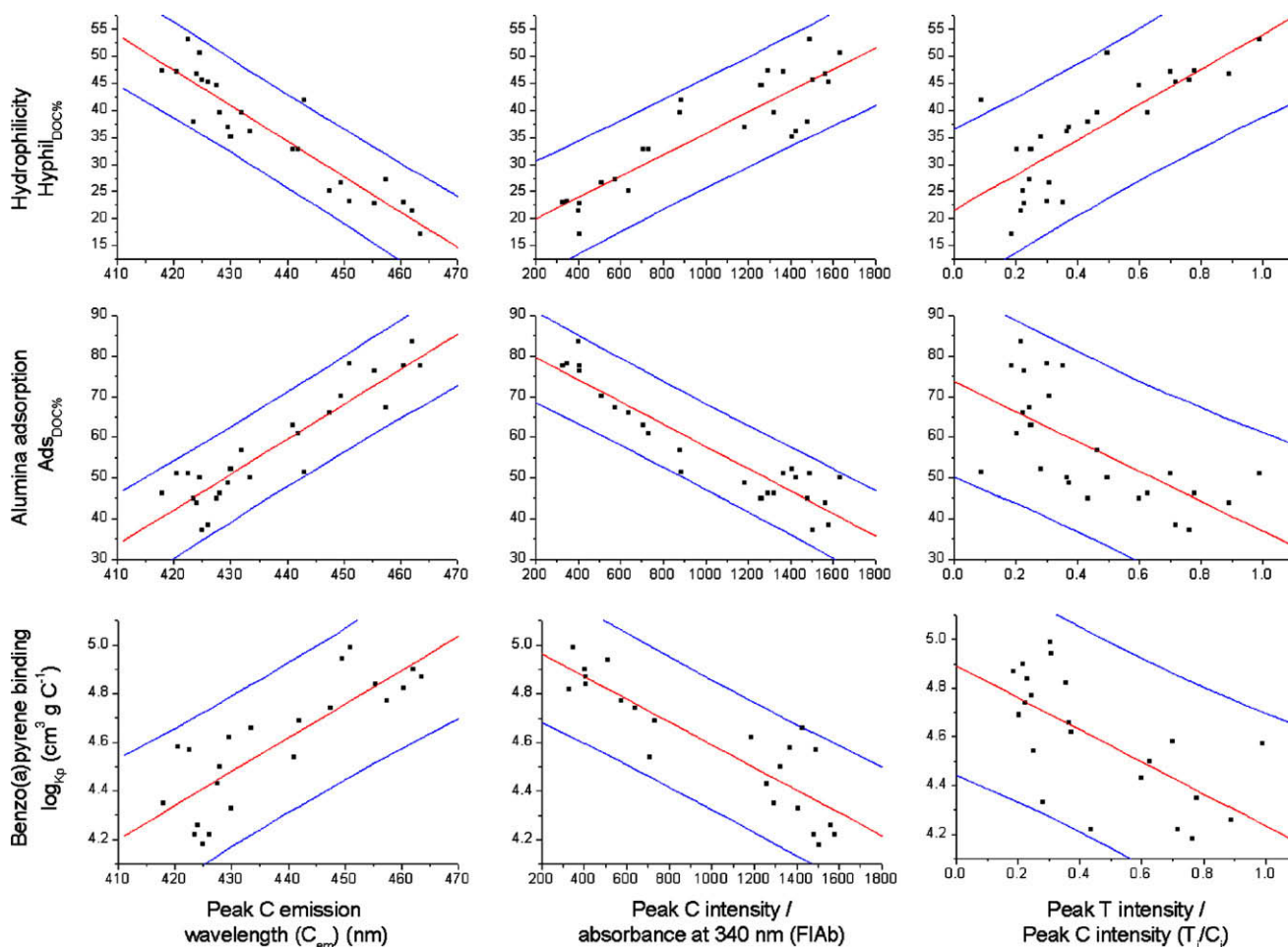
Fig. 2b and c plots some of the fluorescence properties that have been previously shown to best discriminate DOM from different sources. Fig. 2b plots the peak C fluorescence emission wavelength against the ratio of peak T to peak C fluorescence. Fig. 2b shows

that the DOM samples fall on a trend line of high (low) peak T/peak C ratio and low (high) peak C emission wavelength. Peak C emission wavelength has been demonstrated to increase with an increase in the number of highly substituted aromatic nuclei and conjugated unsaturated systems in soil samples (Senesi et al., 1991) and freshwater DOM (Kalbitz et al., 1999). The ratio of peak T to peak C characterizes the relative proportion of fresh organic material; this ratio could therefore be hypothesised to be an indicator of labile vs. recalcitrant DOM. Fig. 2b therefore suggests that samples with the highest biological activity have a lower aromaticity. Within the dataset, Esthwaite Water samples have the lowest

**Table 2**  
Correlations between fluorescence parameters and functional assays (Spearman's rho)

	A <sub>340</sub> loss% 4	A <sub>C4-8</sub> 5	logK <sub>c</sub> 6	logK <sub>p</sub> 7	Hyphil <sub>DOC%</sub> 8	Hyphil <sub>Abs</sub> 9	Ads <sub>doc%</sub> 10	Ads <sub>340</sub> 11
a <sub>340</sub> (/cm)	0.13	<b>0.68</b>	0.01	<b>0.82</b>	<b>-0.93</b>	<b>-0.67</b>	<b>0.89</b>	<b>0.90</b>
Fluorescence:absorbance ratio (FIAb)	-0.07	-0.48	0.11	<b>-0.88</b>	<b>0.86</b>	<b>0.66</b>	<b>-0.88</b>	<b>-0.83</b>
Peak A excitation wavelength (A <sub>ex</sub> )	<b>-0.60</b>	0.08	-0.47	0.05	-0.16	0.05	0.08	0.15
Peak A emission wavelength (A <sub>em</sub> )	-0.15	<b>0.58</b>	-0.16	<b>0.76</b>	<b>-0.65</b>	<b>-0.56</b>	<b>0.64</b>	<b>0.59</b>
Peak A intensity (A <sub>i</sub> )	-0.05	-0.01	0.25	<b>-0.65</b>	0.46	<b>0.54</b>	<b>-0.63</b>	<b>-0.51</b>
Peak C excitation wavelength (C <sub>ex</sub> )	0.09	<b>0.55</b>	-0.12	<b>0.80</b>	<b>-0.90</b>	<b>-0.73</b>	<b>0.80</b>	<b>0.83</b>
Peak C emission wavelength (C <sub>em</sub> )	0.21	<b>0.69</b>	0.08	<b>0.81</b>	<b>-0.92</b>	<b>-0.72</b>	<b>0.85</b>	<b>0.84</b>
Peak C intensity (C <sub>i</sub> )	-0.26	0.27	0.12	-0.46	0.24	0.43	-0.42	-0.29
Peak T intensity (T <sub>i</sub> )	-0.10	<b>-0.66</b>	0.08	<b>-0.82</b>	<b>0.83</b>	<b>0.78</b>	<b>-0.85</b>	<b>-0.86</b>

Statistically significant relationships at the 99.5% confidence level are shown in bold.



**Fig. 3.** Selected DOM fluorescence–function relationships. Linear regression with 95% prediction lines shown. (Left) Peak C emission wavelength (middle) peak C intensity/absorbance at 340 nm and (right) peak T intensity/peak C intensity regressed against (top) hydrophilicity (middle) alumina adsorption and (base) benzo[a]pyrene binding.

peak C emission wavelength and highest peak T/peak C ratio; they also exhibit a seasonal variation with lowest peak C emission wavelength and highest peak T/peak C ratio in summer. When compared to the SRFA standard, the DOM samples do not have strongly similar characteristics. Although SRFA has a similar peak C emission wavelength to the riverine DOM samples, it has lower peak T/peak C ratio.

Fig. 2c presents peak C emission wavelength against peak C fluorescence per unit absorbance. The fluorescence: absorbance ratio has been long recognized as an indicator of DOM molecular weight, with DOM of lower weight having a greater fluorescence per unit absorbance at ~340 nm (Stewart and Wetzel, 1980; Belzile and Guo, 2006; Lead et al., 2006). Fig. 2c shows that the DOM samples cluster into two groups: Esthwaite Water samples having high fluorescence: absorbance ratio and lowest peak C emission wavelength, Rough Sike samples have a low fluorescence: absorbance ratio and high peak C emission wavelength, with other sites falling intermediately to these groups. In comparison with Fig. 2b, little variability is visible within the fluorescence: absorbance ratio in the Esthwaite Water samples, in contrast to the variation in peak C emission wavelength. SRFA standards have a fluorescence:absorbance ratio that reflects the mean value of the FunVar samples, falling between the riverine and lake samples.

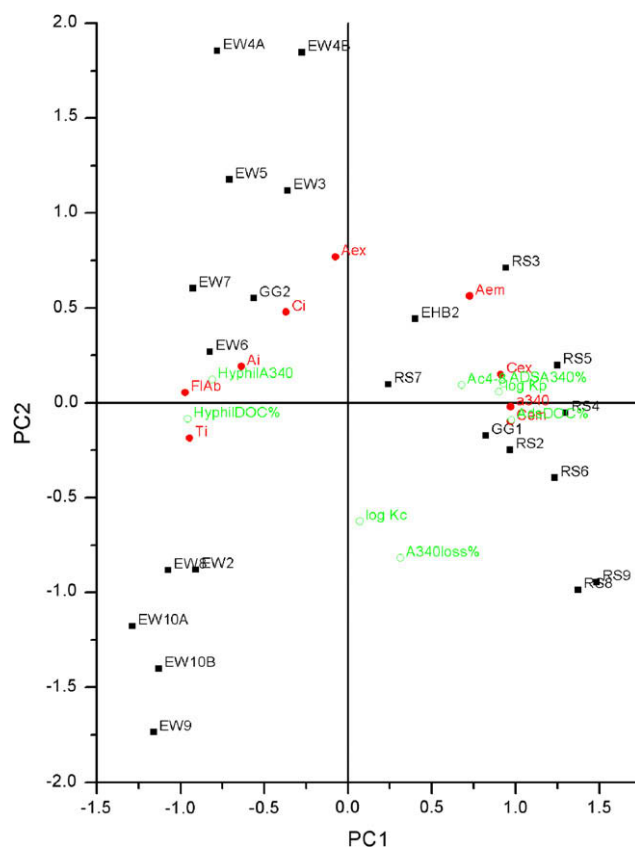
### 3.3. Comparing fluorescence character and DOM function

Spearman's rank correlation coefficients for fluorescence parameters and functional assays are presented in Table 2, selected relationships are shown in Fig. 3. Additionally, stepwise linear regressions were performed for each functional assay against the concentration independent optical parameters (peaks A and C excitation and emission wavelengths, ratios of fluorescence intensity (peak A/C and peak T/C) and the fluorescence:absorbance ratio) (Table 3). In our experimental design, DOM concentration was controlled at 10 mg L<sup>-1</sup>. However, in the natural environment, DOM concentrations will vary, typically between 0.5 and 15 mg L<sup>-1</sup>, and therefore relationships between fluorescence intensity/g C and function properties are likely to be obscured by DOM concentration variations in the natural environment. However, concentration independent fluorescence properties may have more success.

Photochemical fading (assay 4) has only a weak correlation with DOM fluorescence properties. Stepwise linear regression suggests a relatively weak ( $r = -0.54$ ) negative relationship with peak A excitation wavelength. Principal component analysis of the data (Fig. 4) shows that this relationship is strong within the Esthwaite Water dataset, with samples with low fading and high peak A excitation wavelength occurring in Feb–June (EW3–5), and those with high fading and low peak A excitation wavelength in Aug–Nov (EW8–10). The reason for this is at yet unclear, although Gondar et al. (2008) indicate that in late winter and spring the lake is dominated by allochthonous DOM and in summer by autochthonous DOM, suggesting that latter material is more photochemically sensitive.

**Table 3**  
Results of stepwise linear regression models to predict DOM functional assay results from in situ measurable DOM optical properties

Assay number	Linear regression model	r
4	$A_{340} \text{ loss \%} = 442.70 - 1.761A_{\text{ex}}$	-0.54
5	$AC_{4-8} = 5.582 - 3.013T_i/C_i$	0.77
6	No statistically significant model	
7	$\log K_p = 5.057 - 0.000466 \text{ FIAb}$	-0.88
8	$\text{Hyphil}_{\text{DOC}\%} = 322.53 - 0.655C_{\text{em}}$	-0.92
9	$\text{Hyphil}_{\text{Abs}} = 110.7 - 0.21C_{\text{em}}$	-0.76
10	$\text{Ads}_{\text{DOC}\%} = 85.08 - 0.027 \text{ FIAb}$	-0.93
11	$\text{Ads}_{\text{a340}\%} = 98.72 - 0.0246 \text{ FIAb}$	-0.90



**Fig. 4.** Principal component analysis. Samples are as shown squares, functional assay component loadings shown as open circles, and optical properties component loadings shown as closed circles.

Buffering capacity (assay 5) exhibits strongest positive statistically significant relationships with absorbance at 340 nm and fluorescence peak C emission wavelength and a negative relationship with peak T intensity. These fluorescence properties are indicative of DOM that is more aromatic and less microbial in nature. Stepwise regression indicates the strongest correlation with the ratio of peak T to peak C fluorescence intensity ( $r = 0.77$ ). Given that peak C is related to humic-like material, and peak T predominantly to microbial derived matter, this result confirms the relative importance of humic-like material to buffering capacity. In our regression model it suggests that a relative increase in fluorescence peak C intensity predicts a greater buffering potential.

Both benzo[a]pyrene binding (assay 7) and alumina adsorption (assays 10 and 11) increase with both decreasing fluorescence: absorbance ratio and peak T fluorescence intensity; and with increasing peak C excitation and emission wavelengths and absorbance at 340 nm. Binding and adsorption therefore favors DOM with a smaller algal or microbial contribution, higher molecular weight and increased aromaticity. Hydrophilicity (assays 8 and 9) has the inverse relationship with fluorescence parameters to benzo[a]pyrene binding and alumina adsorption, with a strong positive correlation with peak T intensity and the fluorescence:absorbance ratio, and a negative correlation with peak C excitation and emission wavelengths.

Principal component analysis (Fig. 4) of the combined fluorescence and functional assay data shows that a positive score on the first principal component is DOM that has low fluorescence:absorbance ratio, high peak C emission and excitation wavelengths, low peak T intensity, low hydrophilicity and high alumina adsorption, buffering capacity and high benzo[a]pyrene binding.

Rough Sike samples score positively with this component, and Esthwaite Water samples negatively. Principal components analysis confirms the groupings of correlations found in multiple regression modeling. Multiple regression models (Table 3) show that benzo[a]pyrene binding is best modeled by the fluorescence:absorbance ratio ( $r = -0.88$ ); that alumina absorbance is best modeled by the fluorescence:absorbance ratio ( $r = -0.93$  and  $-0.90$ ); and that hydrophilicity is best modeled by fluorescence peak C emission wavelength ( $r = -0.92$  and  $-0.76$ ).

We observe no statistically significant correlations between copper binding (assay 6) and DOM optical properties, although we note that the copper binding assay had a similar pattern of correlations with optical parameters as the photochemical fading assay. Fig. 4 shows that both functional assays plot separately from the DOM optical characteristics.

Given both the speed of fluorescence analysis and the capability to analyze without sample pretreatment; this leads to the potential for rapid and real-time measurement of functional properties. The one caveat would be that previous studies have shown that, in urban rivers at least, significant peak T fluorescence intensity is found in the colloidal and particulate fractions (in contrast with peak C fluorescence, which is predominantly dissolved; Baker et al., 2007). Therefore, the relationship of organic matter function with the fluorescence peak T to peak C intensity ratio of dissolved organic matter, as observed here, might not be as strong in the natural environment. However, in our experiments only buffering capacity correlated with this ratio which might be affected by significant non-dissolved peak T fluorescence in natural waters (Table 3). Therefore, we suggest that the wide temporal and spatial variability of our samples allowed our findings to be applied to other sites with similar catchment characteristics, e.g. rural upland and lowland catchments in temperate maritime climates.

#### 4. Conclusions

Our optical characterization of DOM samples demonstrates that peak C fluorescence emission wavelength, the ratio of peak T to peak C fluorescence intensity, and the fluorescence:absorbance ratio best differentiate different DOM samples. In terms of its fluorescence properties, the SRFA standard is only in part typical of our observed DOM fluorescence characteristics; in particular many DOM samples had a lower peak C emission wavelength (less aromatic) and higher peak T intensity (more algal or microbial derived DOM).

Peak C fluorescence emission wavelength, the ratio of peak T to peak C fluorescence intensity, and the fluorescence:absorbance ratio fluorescence parameters have strong correlations with several of the functional assays, in particular the binding and adsorption assays, and buffering capacity. In many cases, regression equations with a correlation coefficient  $>0.8$  are obtained, suggesting that DOM functional character can be predicted from DOM fluorescence properties.

In our experimental design, DOM concentrations have been held constant, and therefore fluorescence intensity and absorbance are effectively per g C. However, in the natural environment, changing DOM concentration would obscure any variations in fluorescence intensity per g C. Hence, relationships between fluorescence intensity and functional character observed in our controlled experimental conditions are unlikely to be observed in the field. However, Table 3 presents linear regressions where only the concentration independent predictors (fluorescence:absorbance ratio, fluorescence intensity ratios, and concentration independent fluorescence excitation and emission wavelengths) were included in the model. Table 3 shows that the assays for extinction coefficient, photochemical fading, benzo[a]pyrene binding, hydro-

philicity and alumina adsorption could be predicted from these optical properties in the field and independent of concentration (for the range of samples investigated here;  $\text{DOC} < 10 \text{ mg L}^{-1}$  and absorbance at 340 nm  $< 0.4$ ).

Future work could address the relationship between function and OM character for a wider range of organic matter size fractions than just dissolved; to a wider range of aquatic environments to include ground water, drinking water and non-potable recycled water, where dissolved organic matter might be expected to dominate the total organic matter fraction; and to apply our findings to in situ (non-extracted) monitoring of DOM character and function in these environments.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.chemosphere.2008.09.018](https://doi.org/10.1016/j.chemosphere.2008.09.018).

#### References

- Baker, A., 2001. Fluorescence excitation–emission matrix characterisation of some sewage impacted rivers. *Environ. Sci. Technol.* 35, 948–953.
- Baker, A., Elliott, S., Lead, J.R., 2007. Effects of filtration and pH perturbation on organic matter fluorescence. *Chemosphere* 67, 2035–2043.
- Belzile, C., Guo, L., 2006. Optical properties of low molecular weight and colloidal organic matter: application of the ultrafiltration permeation model to DOM absorption and fluorescence. *Mar. Chem.* 98, 183–196.
- Cammack, W.K.L., Kalf, J., Prairie, Y.T., Smith, E.M., 2004. Fluorescent dissolved organic matter in lakes: relationship with heterotrophic metabolism. *Limnol. Oceanogr.* 49, 2034–2045.
- Corvace, M., Zsolnay, A., D'Orazio, V., Lopez, R., Miano, T.M., 2006. Characterization of water extractable organic matter in a deep soil profile. *Chemosphere* 62, 1583–1590.
- Elliott, S., Lead, J.R., Baker, A., 2006. Characterisation of the fluorescence from freshwater, planktonic bacteria. *Water Res.* 40, 2075–2083.
- Gjessing, E.T., Egeberg, P.K., Harkedal, J., 1999. Natural organic matter in drinking water—the “NOM typing project”, background and basic characteristics of original water samples and NOM isolates. *Environ. Int.* 25, 145–159.
- Gondar, D., Thacker, S.A., Tipping, E., Baker, A., 2008. Functional variability of dissolved organic matter from a productive lake. *Water Res.* 42, 81–90.
- Heal, O.W., Smith, R.A.H., 1978. *Production Ecology of British Moors and Montane Grasslands*. Springer-Verlag.
- Hessen, D.O., Tranvik, L.J., 1998. *Aquatic Humic Substances*. Springer, Berlin.
- Hudson, N.J., Baker, A., Ward, D., Brunson, C., Reynolds, D., Carliell-Marquet, C., Browning, S., 2008. Fluorescence spectrometry as a surrogate for the BOD<sub>5</sub> test in water quality assessment: an example from South West England. *Sci. Total Environ.* 391, 149–158.
- Hudson, N.J., Baker, A., Reynolds, D., 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters – a review. *Rivers Res.* 23, 631–649.
- Kalbitz, K., Geyer, W., Geyer, S., 1999. Spectroscopic properties of dissolved humic substances – a reflection of land use history in a fen area. *Biogeochemistry* 47, 219–238.
- Kullberg, A., Bishop, K.H., Hargeby, A., Jansson, M., Petersen, R.C., 1993. The ecological significance of dissolved organic carbon in acidified waters. *Ambio* 22, 331–337.
- Lead, J.R., De Momi, A., Goula, G., Baker, A., 2006. Fractionation of freshwater colloids and particles by SPLITT: analysis by electron microscopy and 3D excitation–emission matrix fluorescence. *Anal. Chem.* 78, 3609–3615.
- Mobed, J.J., Hemmingsen, S.L., Autry, J.L., McGown, L.B., 1996. Fluorescence characterisation of IHSS Humic substances: total luminescence spectra with absorbance correction. *Environ. Sci. Technol.* 30, 3061–3066.
- Nguyen, M.-L., Westerhoff, P.E., Baker, L., Hu, Q., Esparza-Soto, M., Sommerfeld, M., 2005. Characteristics and reactivity of algae-produced dissolved organic carbon. *J. Environ. Eng.* 131, 1574–1582.
- Ohno, T., 2002. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environ. Sci. Technol.* 36, 742–746.
- Perdue, E.M., Gjessing, E.T., 1990. *Organic Acids in Aquatic Ecosystems*. Wiley, New York.
- Ramsbottom, A.E., 1976. *Depth Charts of the Cumbrian Lakes*. Freshwater Biological Association.



- Senesi, N., Miano, T.M., Provenzano, M.R., Brunetti, G., 1991. Characterization, differentiation and classification of humic substances by fluorescence spectroscopy. *Soil Sci.* 152, 259–271.
- Stewart, A.J., Wetzel, R.G., 1980. Asymmetrical relationships between absorbance, fluorescence, and dissolved organic carbon. *Limnol. Oceanogr.* 26, 590–597.
- Thacker, S.A., Tipping, E., Baker, A., Gondar, D., 2005. Development and application of functional assays for freshwater dissolved organic matter. *Water Res.* 39, 4559–4573.
- Thacker, S.A., Tipping, E., Gondar, D., Baker, A., in press. Functional properties of DOM in a stream draining blanket peat. *Sci. Total Environ.*
- Urban-rich, J., McCarty, J.T., Fernández, D., Acuña, J.L., 2006. Larvaceans and copepods excrete fluorescent dissolved organic matter (FDOM). *Journal of Experimental Marine Biology and Ecology* 332, 96–105.