
Spectrophotometric discrimination of river dissolved organic matter

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Abstract:

There is a need to be able to differentiate the dissolved organic matter (DOM) fraction in river waters. Research in the 1970s and 1980s has attempted to utilize both absorbance and fluorescence to distinguish between DOM fractions in river waters, but both were limited by the available technology. Total organic carbon content has, therefore, been widely used as a standard method of measuring DOM concentration, although it has little power to differentiate DOM fractions. Recent advances in fluorescence spectrophotometry enable rapid and optically precise analysis of DOM. Here, we show how a combination of both fluorescence and absorbance can be used to discriminate statistically between spatial variations of DOM in tributaries in a small catchment of the Ouseburn, NE England. The results of the discriminant analysis suggest that about 70% of the samples can be correctly classified to its tributary. Discriminant function 1 explains 60.8% of the variance in the data and the fulvic-like fluorescence intensity has the largest absolute correlation within this function; discriminant function 2 explains a further 21.5% of the variance and the fulvic-like fluorescence emission wavelength has the largest absolute correlation within this function. The discriminant analysis does not correctly classify all tributaries every time, and successfully discriminates between the different tributaries 70% of the time. Occasions when the tributary waters are less well discriminated are due to either episodic pollution events (at two sites) or due to tributaries that have strong seasonal trends in spectrophotometric parameters, which allows the sites to be misclassified. Results suggest that spectrophotometric techniques have considerable potential in the discrimination of DOM in rivers. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS dissolved organic matter; total organic carbon; fluorescence; absorption; spectrophotometry

INTRODUCTION

Total organic carbon (TOC) content has been widely used as a standard method for measuring river dissolved organic matter (DOM) (Tipping *et al.*, 1997), although it has little discriminatory power as it cannot distinguish between carbon fractions. There is a need, however, to be able to discriminate between different DOM sources in river water. For example, it would then be possible to use DOM as a natural tracer (Lobbess *et al.*, 2000), and to monitor and detect organic pollutants (Siepak, 1999). If discrimination of different DOM sources is possible, this might help improve our understanding of the chemical and structural processes generating these variations in DOM properties. Understanding DOM properties and their interactions in the environment would lead to improved understanding of DOM function, such as proton binding, metal ion, pesticide and polycyclic aromatic hydrocarbon interactions (Jordan *et al.*, 1997, MacDonald *et al.*, 1997) and the effect of DOM on ecosystem functions (Band *et al.*, 2001).

Spectrophotometric techniques, such as fluorescence and absorption, have the potential to discriminate between DOM fractions, particularly to differentiate the labile fraction into environmentally significant components. Early research attempted to utilize both absorbance (Sheppard, 1977; Grieve, 1985) and fluorescence (Wilander *et al.*, 1974; Smart *et al.*, 1976), but both were limited by the available technology.

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However, recent technological advances have led to a rapid analysis time that permits the near real-time monitoring of river waters. Rapid (~ 1 min) detection of river water fluorescence in small (< 5 ml) samples at concentrations of $\sim 0.1 \text{ mg l}^{-1}$ is now possible (Baker, 2001, 2002). Figure 1 shows the location of these fluorescence centres in optical space. The principal fluorophores are ascribed to aromatic and aliphatic groups in humic and fulvic substances and to aromatic proteins (Coble, 1996; Mayer *et al.*, 1999; Baker, 2001). The presence of high intensities of tryptophan fluorescence (T_{int}) has been used to identify sewage-related DOM in treatment plants and river water (Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Baker, 2001). Sewage-impacted rivers were characterized by both high fulvic-like F_{int} and tryptophan T_{int} fluorescence intensity (Baker, 2001), and it was demonstrated that fluorescence quenching by metal ions was not important, due to the high concentration of organic matter in the wastewater. Farm wastes (sheep barn waste, silage liquor, pig and cattle slurry) also have distinctive fluorescence (Baker, 2002) and absorption properties (Brookman, 1997) that might also impact on river fluorescence properties in agricultural catchments.

Recent advances in fluorescence spectrophotometry have, therefore, started to discriminate between DOM from different sources; however, research to date has focused on laboratory experimentation or simplistic fluvial systems, e.g. reaches up and downstream of sewerage inputs. The time is therefore right for further research to investigate the variability of river fluorescence properties in a more complex system of a small catchment that potentially has a wider variety of DOM sources. In addition, a wider range of pH and metal-ion concentrations may be experienced, which might interfere with any fluorescence–DOM relationships. River water pH can affect both fluorescence intensity and wavelength (Mobed *et al.*, 1996), although laboratory studies suggest that fulvic-like fluorescence intensity will vary by less than 10% in the pH range 7.0 to 8.4 (Miano *et al.*, 1988). More important may be fluorescence quenching by certain metal ions (such as Cu^{2+} , Co^{2+} , Cr^{3+} , Cr^{4+} , Fe^{2+} , Fe^{3+} , Mn^{2+} , Ni^{2+} , Pb^{2+}) and both quenching and enhancing of fluorescence by Al^{3+} . Metal–DOM interactions have been thoroughly modelled (Tipping, 1998) and fluorescence quenching and wavelength shifts have been observed in laboratory experiments (da Silva *et al.*, 1998; Elkins and Nelson,

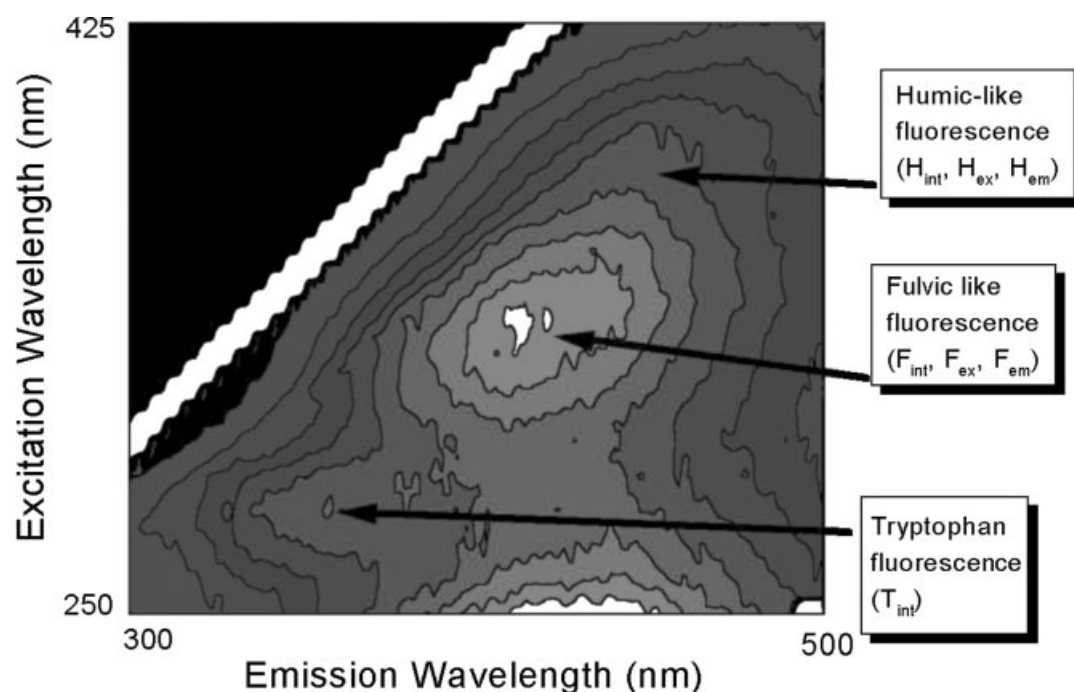


Figure 1. Typical fluorescence excitation–emission matrix showing the locations of the fluorescence peaks analysed in this paper

2001). The latter demonstrate that a $\times 2$ quenching/enhancing of fulvic-like fluorescence intensity is possible at metal-ion concentrations up to 1 mmol l^{-1} . Therefore, it is of interest to investigate if a combination of spectrophotometric techniques, both fluorescence and absorption, can be used to discriminate DOM from different input tributaries in the more complex environment of a small catchment.

METHODS

Description of study area

The small catchment chosen for our research is the Ouseburn, NE England, which is 55 km^2 in area. Figure 2 shows the catchment and the water sampling locations from which samples were collected between June 2000 and November 2001. Sample locations and numbering follow that of Turnball and Bevan (1994) and incorporate the UK Environment Agency chemical water quality sample sites on the river. The lower catchment (downstream of site 13) is urbanized, as it flows through the city of Newcastle-upon-Tyne; arable farms (upstream of site 13) and the city international airport (runoff draining into sites 6 and 8) dominate the upper catchment. The catchment has a long history of research; integrated water quality monitoring on the river found it adversely affected by combined sewer overflows, cross-connected sewers and airport de-icer (Turnball and Bevan, 1994). Spatial variations in dissolved and particulate lead derived from the region's industrial legacy have also been analysed (Mellor and Bevan, 1999).

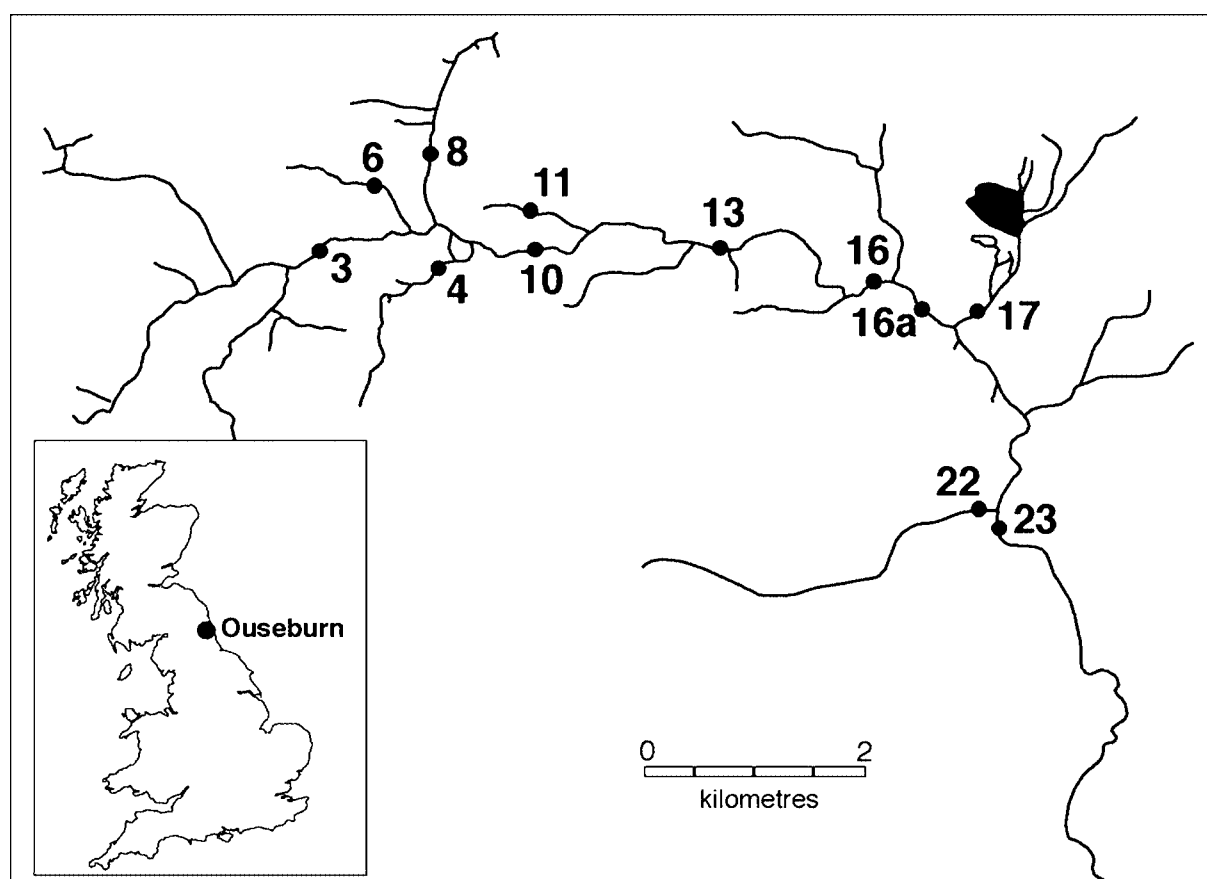


Figure 2. The Ouseburn catchment, Newcastle-upon-Tyne, NE England ($55^\circ 0' \text{ N}$, $1^\circ 35' \text{ W}$), showing sampling locations

In addition to the previous research on the river, chemical water quality data were collected over the study period by both the author and the Environment Agency. Environment Agency data collected as part of the UK national Chemical Water Quality Classification assigns the chemical water quality of the river as Fair to Fairly Good. Monthly samples were collected from January 1999 to July 2001 (with a data gap from February to June 2001 due to access limitations) at sites 23, 16, 10 and 3. The mean biological oxygen demand, ammoniacal nitrogen and dissolved oxygen are respectively 2.9 mg l^{-1} , 0.16 mg l^{-1} and 93.0% at site 23, 3.0 mg l^{-1} , 0.14 mg l^{-1} and 94.1% at site 16, 4.4 mg l^{-1} , 0.11 mg l^{-1} and 89.5% at site 10, and 2.1 mg l^{-1} , 0.16 mg l^{-1} and 83.5% at site 3. Basic chemical data (pH, conductivity) collected by the author for all the sample sites over the study period are presented in Table I. The mean pH ranges from a minimum of 7.46 ± 0.48 (site 8) to a maximum of 7.80 ± 0.25 (site 22). All sites have a coefficient of variation of $<10\%$ and there is no significant difference between sites (Student's *t*-test, 95% confidence level), suggesting that pH effects on spectrophotometric properties will not be significant. The pH at most sites has a slight winter maximum, which correlates with the highest electrical conductivity ($(2-4) \times 10^3 \mu\text{S}$). Electrical conductivity exhibits a greater spatial and temporal variability within the catchment than pH. The lowest electrical conductivity is observed at site 17 ($562 \pm 105 \mu\text{S}$) and the highest at site 6 ($1368 \pm 839 \mu\text{S}$). Electrical conductivity is highest in winter after periods of frost and/or snow cover, and correlates strongly with chloride concentration ($r = 0.97$, $n = 70$, data not presented), suggesting the importance of road salt as a pollutant in the catchment. Environment Agency metal-ion data for the study period (site 23; 12 samples) confirms that other dissolved metal concentrations are less than 5 mg l^{-1} (Pb: $0.32 \pm 0.44 \mu\text{g l}^{-1}$; Zn: $4.06 \pm 3.77 \mu\text{g l}^{-1}$; Cr: $0.79 \pm 0.61 \mu\text{g l}^{-1}$; Ni: $<5 \mu\text{g l}^{-1}$; Cu: $5.16 \pm 1.80 \mu\text{g l}^{-1}$; Fe: $129.2 \pm 147.8 \mu\text{g l}^{-1}$; V: $1.34 \pm 1.00 \mu\text{g l}^{-1}$).

Sampling protocol

A spatial sampling programme was undertaken monthly on all accessible major tributaries (seven sites: 3, 4, 6, 8, 11, 17, 22), together with intermediate main stream samples (five sites: 10, 13, 16, 16a, 23). One site, the airport tributary (site 8), was not sampled in April and May 2001 due to access limitations. Mean river discharge over the study period measured at the Environment Agency gauging station at Crag Hall (site 23) was $0.39 \text{ m}^3 \text{ s}^{-1}$ (15 min sampling frequency; median $0.17 \text{ m}^3 \text{ s}^{-1}$, max $17.0 \text{ m}^3 \text{ s}^{-1}$, min $0.025 \text{ m}^3 \text{ s}^{-1}$). Water samples were collected in 30 ml polypropylene bottles that had been precleaned in 10% HCl and distilled water. Samples were refrigerated upon return from the field, and analysed within 24 h. The samples were filtered (Whatman GF/C preashed glass microfibre filter papers). Fluorescence measurements were undertaken using a Perkin–Elmer LS-50B luminescence spectrometer as described elsewhere (Baker, 2001; Baker and Lamont-Black, 2001). The Raman peak of water at 348 nm was used as a test for machine stability and to permit inter-laboratory comparison, and results were adjusted to a Raman peak intensity of 20.0 units. Absorption at 254 nm, 340 and 410 nm was undertaken using a WPA Lightwave UV–VIS spectrometer; these wavelengths were chosen as they have historically been utilized as an indication of sewage, of the wavelength of absorption of fulvic-like material and of the standard absorption wavelength for colour respectively. Absorption correction was not applied to the fluorescence spectra (Mobed *et al.*, 1996); absorption of $0.3-0.5 \text{ cm}^{-1}$ at 254 and 340 nm decreased tryptophan and fulvic-like fluorescence intensities by $<10\%$. TOC was determined using a Shimadzu 5000 TOC analyser; all samples were analysed in duplicate or triplicate and the mean value used.

RESULTS

Results for the seven tributaries and six mainstream samples taken at monthly intervals over the period June 2000 to November 2001 are presented in Table I. One of the first considerations is to check for any fluorescence quenching due to pH or metal binding. No correlation (Spearman's rho, 95% confidence level) is

Table I. Summary spectrophotometric and organic carbon data

| Site | pH | Electrical conductivity ($\mu\text{S cm}^{-1}$) | Carbon analysis (mg l^{-1}) | | | Absorption (cm^{-1}) | | | Tryptophan fluorescence intensity T_{int} (U) | Fulvic-like fluorescence | | | Humic-like fluorescence | | |
|------|-------------------------|---|--|-------|--------|---------------------------------|----------------------|----------------------|--|--|--|--------------------------------|--|--|--------------------------------|
| | | | TC | IC | TOC | $A_{254 \text{ nm}}$ | $A_{340 \text{ nm}}$ | $A_{410 \text{ nm}}$ | | Excitation wavelength F_{ex} (nm) | Emission wavelength F_{em} (nm) | Intensity F_{int} (U) | Excitation wavelength F_{ex} (nm) | Emission wavelength F_{em} (nm) | Intensity H_{int} (U) |
| 3 | mean 7.67 stdev 0.33 | 890 | 53.43 | 46.25 | 7.18 | 0.139 | 0.039 | 0.013 | 66 | 331.4 | 418.4 | 133 | 377.1 | 468.8 | 66 |
| 4 | mean 7.53 stdev 0.33 | 285 | 5.51 | 6.63 | 3.69 | 0.080 | 0.037 | 0.018 | 25 | 7.4 | 5.7 | 26 | 5.8 | 7.4 | 18 |
| 6 | mean 7.63 stdev 0.38 | 938 | 47.24 | 38.71 | 8.52 | 0.208 | 0.057 | 0.019 | 134 | 328.2 | 417.0 | 202 | 379.6 | 467.4 | 99 |
| 8 | mean 7.46 stdev 0.48 | 639 | 11.94 | 8.96 | 4.34 | 0.109 | 0.039 | 0.020 | 160 | 6.1 | 2.9 | 76 | 6.3 | 5.7 | 35 |
| 10 | mean 7.71 stdev 0.52 | 1368 | 59.06 | 48.94 | 10.12 | 0.220 | 0.062 | 0.021 | 114 | 328.2 | 416.4 | 221 | 378.6 | 468.3 | 108 |
| 11 | mean 7.59 stdev 0.44 | 839 | 7.99 | 9.16 | 3.07 | 0.054 | 0.024 | 0.013 | 43 | 6.4 | 5.1 | 43 | 6.6 | 5.2 | 20 |
| 13 | mean 7.75 stdev 0.30 | 899 | 119.65 | 58.90 | 60.74 | 0.351 | 0.091 | 0.027 | 151 | 324.6 | 417.3 | 319 | 381.7 | 468.7 | 151 |
| 16 | mean 7.74 stdev 0.35 | 367 | 159.65 | 12.55 | 156.25 | 0.054 | 0.018 | 0.012 | 30 | 7.8 | 2.5 | 60 | 2.5 | 5.3 | 33 |
| 16a | mean 7.64 stdev 0.62 | 981 | 57.90 | 46.25 | 11.65 | 0.177 | 0.052 | 0.019 | 88 | 328.8 | 417.3 | 165 | 378.8 | 820.3 | 83 |
| 17 | mean 7.59 stdev 0.37 | 675 | 13.48 | 7.03 | 12.16 | 0.069 | 0.032 | 0.016 | 37 | 7.7 | 6.4 | 32 | 6.1 | 1217.9 | 22 |
| 22 | mean 7.80 stdev 0.25 | 870 | 55.10 | 46.43 | 8.67 | 0.257 | 0.108 | 0.051 | 44 | 330.8 | 419.7 | 136 | 378.1 | 465.0 | 78 |
| 23 | mean 7.75 stdev 0.32 | 595 | 12.96 | 18.01 | 5.24 | 0.283 | 0.156 | 0.090 | 17 | 9.5 | 7.1 | 70 | 4.8 | 8.5 | 43 |
| | | 990 | 55.37 | 46.18 | 9.19 | 0.173 | 0.054 | 0.023 | 84 | 330.0 | 416.4 | 164 | 378.1 | 467.6 | 82 |
| | | 695 | 6.30 | 7.52 | 4.26 | 0.093 | 0.055 | 0.043 | 32 | 6.1 | 3.4 | 26 | 3.8 | 6.6 | 17 |
| | | 970 | 53.30 | 44.52 | 8.77 | 0.168 | 0.052 | 0.021 | 85 | 329.3 | 417.5 | 155 | 378.5 | 468.3 | 77 |
| | | 666 | 5.87 | 7.11 | 4.14 | 0.081 | 0.044 | 0.028 | 24 | 6.5 | 4.3 | 26 | 7.2 | 6.2 | 17 |
| 16a | mean 7.64 stdev 0.62 | 1069 | 55.01 | 45.91 | 9.10 | 0.173 | 0.059 | 0.029 | 80 | 326.9 | 417.6 | 155 | 378.8 | 468.0 | 76 |
| 17 | mean 7.59 stdev 0.37 | 785 | 5.10 | 7.91 | 4.94 | 0.125 | 0.076 | 0.057 | 21 | 7.5 | 5.6 | 32 | 5.8 | 6.2 | 21 |
| 22 | mean 7.80 stdev 0.25 | 562 | 43.21 | 30.18 | 13.03 | 0.410 | 0.132 | 0.047 | 87 | 332.7 | 429.3 | 232 | 379.0 | 470.9 | 125 |
| 23 | mean 7.75 stdev 0.32 | 105 | 3.81 | 4.09 | 2.00 | 0.092 | 0.053 | 0.033 | 76 | 8.0 | 13.7 | 53 | 6.0 | 5.4 | 15 |
| | | 908 | 64.36 | 55.52 | 8.84 | 0.213 | 0.059 | 0.017 | 75 | 331.3 | 417.9 | 193 | 379.3 | 469.0 | 100 |
| | | 273 | 18.59 | 22.86 | 5.44 | 0.140 | 0.050 | 0.018 | 19 | 6.4 | 6.4 | 34 | 5.9 | 5.2 | 29 |
| | | 893 | 50.95 | 41.66 | 9.29 | 0.211 | 0.061 | 0.024 | 84 | 329.7 | 418.0 | 178 | 379.0 | 469.1 | 88 |
| | | 449 | 4.68 | 6.45 | 2.70 | 0.109 | 0.055 | 0.042 | 25 | 8.3 | 6.3 | 23 | 3.9 | 4.8 | 14 |

observed between pH and F_{int} , either for the dataset as a whole ($r = -0.19$) or for individual tributaries. Using electrical conductivity data as a proxy for metal quenching effects, a similar lack of correlation is observed for the dataset as a whole ($r = -0.17$). One tributary (site 4) has a statistically significant correlation (95% confidence level, $r = -0.68$), with a low fluorescence intensity F_{int} observed in January 2001 when electrical conductivity was recorded at 3060 μS . Further research is needed to assess if this is indicating a quenching effect of metal ions associated with road salt at this time or whether it is a non-causal correlation based on one outlying data point.

Figure 3 presents contrasting spectrophotometric and organic carbon data from the seven tributaries. Figure 3a presents TOC; the highest values are observed at site 8, which takes run-off from Newcastle International Airport and includes the de-icer propylene glycol. However, most tributaries are statistically indistinguishable due to high range of TOC between 5 and 20 mg l^{-1} at most sites, with only site 17 being statistically higher than sites 3, 6, 11, and 22 (Student's t -test, 95% confidence level). Figure 3b presents fluorescence/TOC, which quantifies fluorescence per unit carbon and might be expected to differentiate DOM

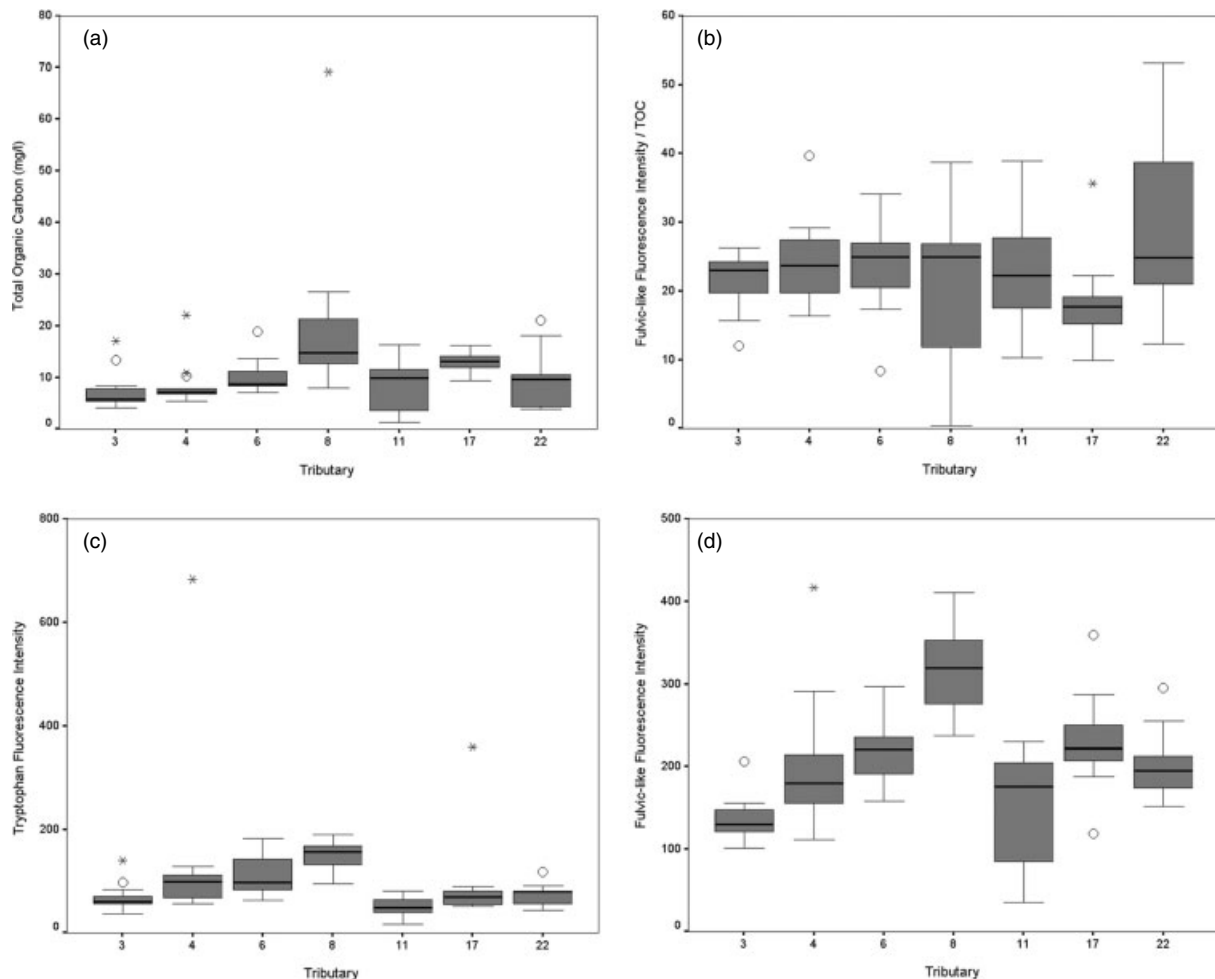


Figure 3. Boxplots of (a) TOC, one outlier on site 8 at 555 mg l^{-1} is omitted for clarity, (b) $F_{\text{int}}/\text{TOC}$, (c) T_{int} , and (d) F_{int}° and * represent outliers (cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box) and extremes (cases with values greater than three box lengths from the upper or lower edge of the box) respectively

properties in the catchment. However, tributary values are similar, with only site 17 statistically lower than sites 4, 6 and 22 (Student's *t*-test, 95% confidence interval). Both high TOC and low fulvic-like fluorescence per gram carbon, therefore, distinguish site 17; this tributary is the only one with a large amount of woodland, as well as containing a small lake (Figure 2). The TOC and fluorescence values could, therefore, be providing information about DOM properties unique to each of these catchment characteristics.

Figure 3c and d presents two spectrophotometric parameters, the fluorescence intensities of the tryptophan T_{int} and fulvic-like F_{int} fluorescence centres. T_{int} is lowest at sites 3 and 11, both of which are agricultural tributaries that appear to have no significant DOM input from farm wastes (Baker, 2002). T_{int} is highest at sites 6 and 8; both these sites are adjacent to Newcastle International Airport, which, for an unknown reason, appears to provide a source of high T_{int} DOM. Statistically, river water T_{int} can differentiate between many tributaries (Student's *t*-test, 95% confidence level), with statistically similar sites being 3 and 4; 17 and 22; 4 and 6/8/17; and 22 and 6/17. No statistically significant (95% confidence level, Spearman's rho) correlation is observed between T_{int} and TOC for all the data combined ($n = 91$, $r = 0.20$). A similar lack of correlation is observed when each of the tributaries is considered individually (individual r values range between -0.37 and 0.20), which helps explain the more marked differences between tributaries in terms of T_{int} (Figure 3c) than TOC (Figure 3a). Figure 3d presents F_{int} data for the seven tributaries. Unlike the T_{int} –TOC relationship, a statistically significant (99% confidence level, Spearman's rho) correlation is observed between F_{int} and TOC for the data as a whole ($r = 0.68$). In this case, this hides a strong variability between the relationship between F_{int} and TOC on individual tributaries, with statistically significant (95% confidence level; $r > 0.5$) correlations for sites 3, 11 and 22, explaining at least some of the visual similarity between Figure 3a and d. The correlation is weakest for sites 6 ($r = 0.05$) and 8 ($r = -0.32$), both sites adjacent to Newcastle International Airport and whose DOM fraction includes non-fluorescent propylene glycol de-icer. Statistically (Student's *t*-test, 95%), F_{int} differentiates site 8 from all other sites, site 3 from all sites except 11, and cannot separate sites 4, 6, 17 and 22 at this confidence level.

Temporal variations are presented in Figure 4 for the spectrophotometric parameters F_{int} and T_{int} , together with TOC and river discharge from the Environment Agency gauging station at the downstream site 23. Figure 4b shows that TOC has some seasonal variability, with most tributaries having highest values in the autumn 2000 after the first autumn increase in river discharge. Site 8 (and to a lesser extent site 6) has a later TOC maximum in January and February 2001, due to propylene glycol de-icer applications to the nearby Newcastle International Airport draining into the tributaries after a period of significant snow cover and frost. A TOC peak is also observed on site 4 in the autumn of 2001, after a manhole collapsed into and blocked part of the sewerage system within the catchment and led to untreated sewage entering the tributary. Associated peaks in F_{int} and T_{int} can be seen in Figure 4b and c typical of sewerage pollution (Baker, 2001). Figure 4c demonstrates a greater differentiation between tributaries for F_{int} than for TOC, as already suggested by Figure 3. One site, 11, shows a strong seasonality in F_{int} , and the autumn sewerage impact on site 4 can also be observed. Figure 4d shows a seasonal trend in T_{int} , with the highest values during summer baseflow, when the river has a greater proportion of flow from failing combined sewer overflows and from cross-connected sewers. Superimposed on this trend is the point pollution event on tributary 4, the effect on T_{int} of another sewerage failure (a blocked sewer leading to an unconsented combined sewerage overflow) upstream of site 3 in November 2001, and an unsourced high T_{int} event on site 17 in October 2001.

Figures 3 and 4 show that both spatial and temporal variations are observed in TOC and fluorescence properties, several of which can be attributed to DOM properties. However, graphical representations provide only *ad hoc* information; given the large number of spectrophotometric and carbon parameters (12: T_{int} , F_{em} , F_{ex} , F_{int} , H_{em} , H_{ex} , H_{int} , IC, TOC, A_{254} , A_{340} , A_{410}), and their permutations, a more objective method is required to understand their use in differentiating DOM from the different tributaries. Of particular interest is the ability of spectrophotometric parameters in discriminating between the DOM of the different tributaries. This can be undertaken statistically using discriminant analysis. The object of discriminant analysis is to allocate an individual to one of n populations or groups on the basis of its measurements or properties x (Mardia *et al.*, 1977). In our case, our individual is a point water sample, which we wish to allocate to one

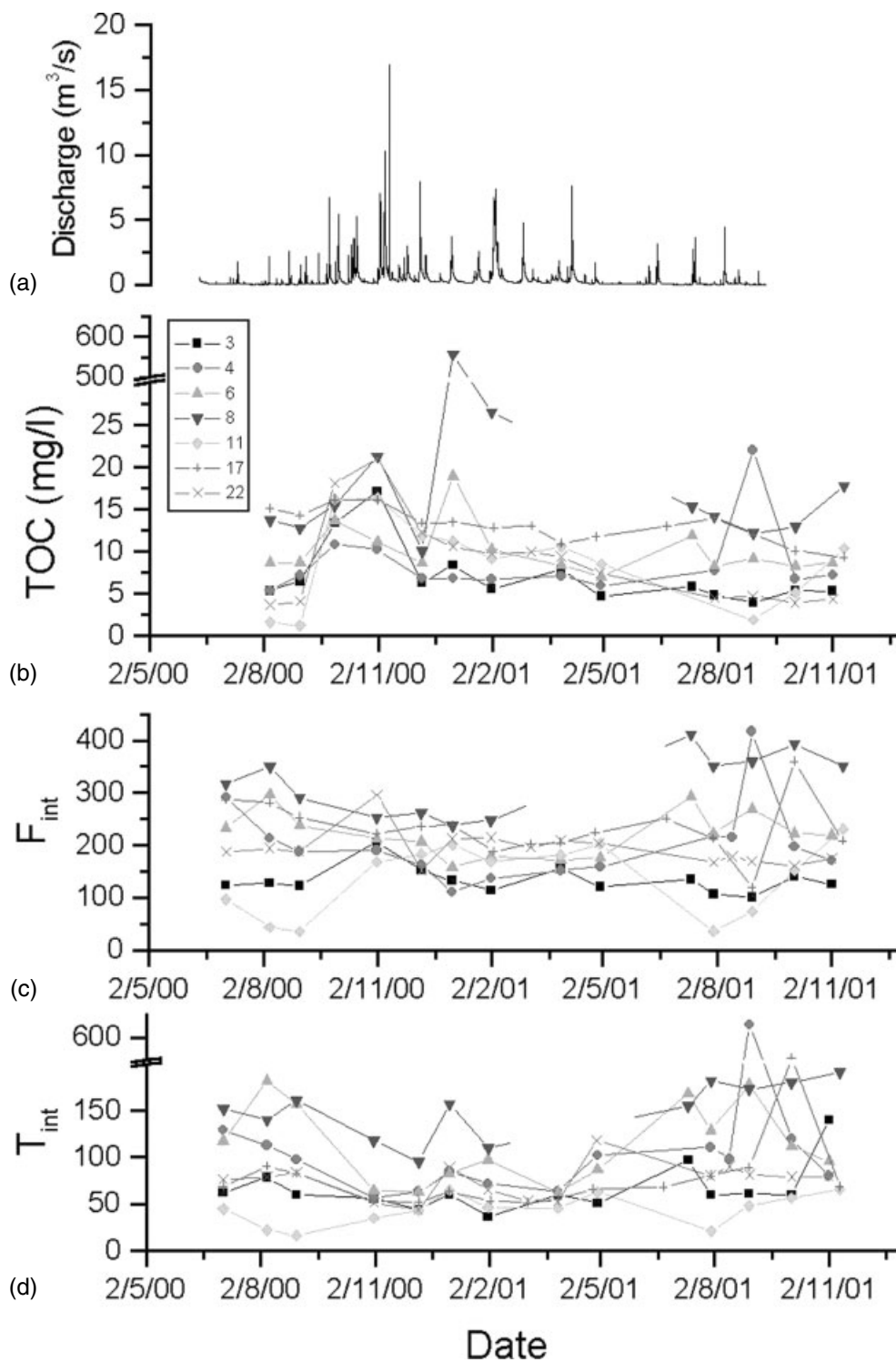


Figure 4. Time series of (a) discharge (Environment Agency gauging station, site 23), (b) TOC, (c) F_{int} , and (d) T_{int}

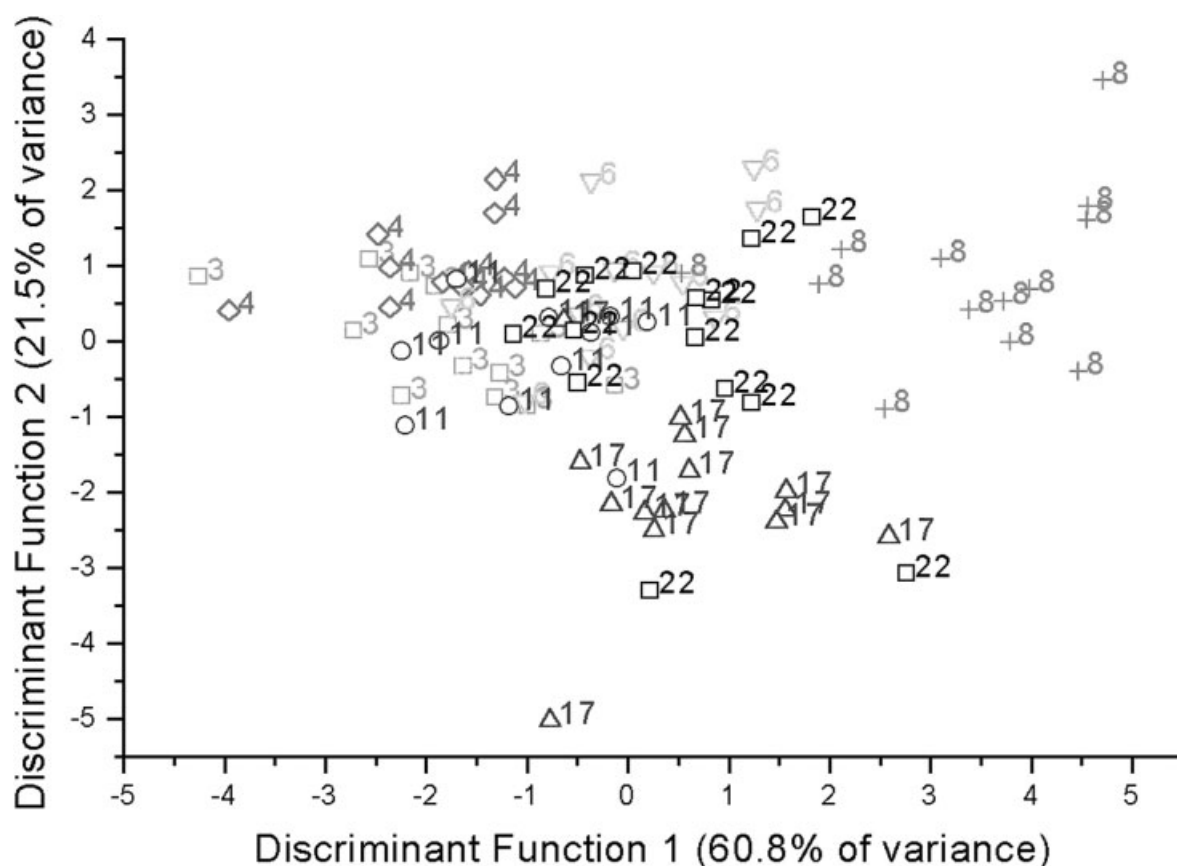


Figure 5. Discriminant analysis functions. Analysis discriminates the different tributaries as follows: site 3 (62%), 4 (75%), 6 (69%), 8 (92%), 11 (46%), 17 (78%), 22 (67%)

of n tributaries (sites 3, 4, 6, 8, 11, 17, 22) where the known properties x are the spectrophotometric and organic carbon measurements. The results of the discriminant analysis suggest that about 70% of the samples can be correctly classified to their tributary. Figure 5 presents plots for the first and second discriminant functions. Discriminant function 1 explains 60.8% of the variance in the data and F_{int} has the largest absolute correlation (0.571) within this function. Site 8 has a particularly high score and is well discriminated by this function due to the high fulvic-like fluorescence intensities on this tributary. Discriminant function 2 explains a further 21.5% of the variance and F_{em} has the largest absolute correlation (-0.580) within this function. Site 17 has a particularly low score and is well discriminated by this function due to its high wavelength of fulvic-like emission. Discriminant function 3 (not graphed) explains a further 11.2% of the variance and IC has the largest absolute correlation (0.538) within this function, the first time that any of the variables related only to TOC analyses (TOC, IC) have discriminatory strength. Discriminant functions 4 to 6 explain a further 4.2%, 1.7% and 0.5% of the data respectively, and have largest absolute correlations with A_{410} , H_{int} and TOC respectively. The discriminant analysis does not correctly classify all tributaries every time and effectively discriminates the different tributaries as follows: site 3 (62%), 4 (75%), 6 (69%), 8 (92%), 11 (46%), 17 (78%), 22 (67%). Occasions when the sites that are less well discriminated are frequently due to the episodic pollution events described earlier. Sites 3 and 4 are misclassified between each other: both sites have suffered from the sewerage pollution events that lead to pulses of high T_{int} and/or F_{int} waters (Figure 4c). Additionally, both have similar F_{int} and F_{em} , and so are poorly discriminated by the first two

discriminant functions (see overlap between data in Figure 5). Sites 11 and 22 have a strong seasonal trend in spectrophotometric parameters (Figure 4b); at site 11 this is due to winter mine water contributions; the cause is unknown at site 22. In each case this seasonal variation allows the site to be misclassified as site 6; Figure 5 shows a strong overlap between these sites, as well as a wide range in discriminant functions 1 and 2.

CONCLUSIONS

Results suggest that spectrophotometric techniques can be used to discriminate DOM between tributaries in the small catchment investigated here. In particular, absorption and fluorescence are both better discriminators than TOC for discriminating organic matter sources; in our discriminant analysis, inorganic carbon and TOC have high correlations in the relatively unimportant third and sixth discriminatory functions respectively. Given the rapid increases in technology, including miniaturization and automation of spectrophotometers, as well as the near-instantaneous analytical time (~ 1 min), spectrophotometry has potential uses for dissolved organic carbon monitoring and pollution control. Further research is needed, including the monitoring of different catchments of varying land use and anthropogenic input. In particular, it is important to understand if DOM discrimination in the Ouseburn is better or worse than in other similarly sized catchments due to the effects of urbanization and its effects on DOM quality. Investigation of DOM spectrophotometric properties in larger catchments is also needed to investigate if discrimination is still possible at this scale.

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