Catchment-scale fluorescence water quality determination

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Abstract

Chemical water quality determinants and river water fluorescence were determined on the River Tyne, northeast England. Statistically significant relationships between nitrate ($r = 0.87$), phosphate ($r = 0.70$), biochemical oxygen demand (BOD) ($r = 0.86$) and dissolved oxygen ($r = -0.65$) and tryptophan-like fluorescence intensity were observed. The strongest correlations are between tryptophan-like intensity and nitrate and phosphate, which in the Tyne catchment derive predominantly from point and diffuse source sewage inputs. The correlation between BOD and the tryptophan-like fluorescence intensity suggests that this fluorescence centre is related to the bioavailable or labile dissolved organic matter pool. The weakest correlations are observed between tryptophan-like fluorescence intensity and ammonia concentration and dissolved oxygen. The weaker correlation with ammonia is due to good ammonia treatment within the wastewater treatment plants within the catchment, and that with dissolved oxygen due to the natural aeration of the river such that this is not a good indicator of water quality. Mean annual tryptophan-like fluorescence intensity, measured by both bench and portable spectrometers, agrees well with the General Water Quality Assessment as determined by the England and Wales environmental regulators, the Environment Agency.

Keywords Water quality; fluorescence; BOD; nitrate; phosphate; DO; ammonia; tryptophan

Introduction

Dissolved organic matter (DOM) has distinctive spectrophotometric properties. As well as strong absorption in ultra-violet light, much DOM fluoresces (FDOM). Recent advances in fluorescent spectrophotometry permit the rapid (~1 min) detection of FDOM at a wide range of both excitation and emission wavelengths to produce an excitation-emission matrix or EEM. An EEM will typically cover a range of excitation and emission wavelengths from ~200 nm (short wavelength UV) through to ~500 nm (visible blue green light), and may contain fluorescence centres that are attributed to both natural DOM groups such as humic and fulvic-like material, as well as fluorescent proteins (Stedmon et al., 2003; Baker, 2001). Studies of DOM EEM fluorescence properties have included wastewater characterisation within the treatment process (Reynolds and Ahmad, 1997; Westerhoff et al., 2001; Vasel and Praet, 2002), DOM characterisation in marine and estuarine waters (Coble et al., 1990; Mopper and Schultz, 1993; Mayer et al., 1999; Parlanti et al., 2001; Stedmon et al., 2003), and riverine DOM fingerprinting (Baker, 2001, 2002a,b; Baker et al., 2003; McKnight et al., 2001, 2003; Thoss et al., 2000; Newson et al., 2001 and Stedmon et al., 2003).

Previous research (Baker, 2001, 2002b) has demonstrated that protein-like fluorescence intensity is increased with increasing anthropogenic DOM inputs from sewerage and farm wastes. Protein-like fluorescence centres observed in EEMs occur at the same locations in optical space as tryptophan and tyrosine standard solutions, and are therefore classified as tryptophan-like and tyrosine-like. Tryptophan-like fluorescence centres occur...
at two wavelength pairs – 220 nm excitation/350 nm emission and 280 nm excitation/350 nm emission, whereas tyrosine-like is predominantly observed at wavelengths of 220 nm excitation/305 nm emission (a second centre at 280 nm excitation is obscured by the Raman line of water). Baker (2002a) and Baker et al. (2003) demonstrate that fluorescence spectrophotometry of these fluorescent protein-like substances can be used to fingerprint DOM sources in a small (~40 km²) urban catchment as well as in environmental monitoring programs through the detection of sewerage pollution events in rivers. Here we seek to determine if a relationship between fluorescence intensity and anthropogenic DOM inputs is maintained in a larger scale (>1,000 km²) catchment, where multiple organic point and diffuse source inputs, together with in-stream organic matter processing, will complicate any distinct fluorescence signature from individual point sources. We also determine if fluorescence properties provide a useful alternative chemical water quality indicator to existing methods (such as biochemical oxygen demand, ammonia, nitrate, phosphate, dissolved oxygen in England and Wales) that are used to determine river water quality. Additionally, we also test the use of a new portable fluorescence spectrometer in the field. Previous research is somewhat limited by the necessity to return samples to the laboratory for analysis, which is both time consuming and can lead to a degradation of water samples prior to analysis. A portable field based spectrophotometer, coupled with the rapidity of the technique (less than 1 minute to obtain a result) would provide field scientists with a rapid pollution monitoring tool, and process control engineers with a portable wastewater monitoring device. Recent technological advances now make this possible, and here we present the first results of river water fluorescence from a range of rivers in northeast England.

Site and samples
The River Tyne in northeast England has a catchment area of 2,935 km² and comprises two main tributaries, the North and South Tyne. The North Tyne rises in the Cheviot Hills near the Scottish Border, the South Tyne in the Cumbrian Pennines. Outside the predominantly rural upland North and South Tyne, approximately 750,000 people live within the rest of the Tyne catchment, and urban and industrial areas have an influence today on the water quality of the river, with 214 consented discharges from sewage treatment works, 126 consented industrial discharges and 492 storm sewer discharges. The Environment Agency classification of the water quality of the river is that 375 km of stream length are of “very good” quality, 204 km are “good”, 17 km are “fairly good”, 23 km are “fair”, 4 km are “poor” and 1 km is “bad”. This overall good water quality has led to the river becoming a major salmon and trout fishery. River lengths with poor quality are predominantly small tributaries in lowland urbanized parts of the catchment with many sewerage and treated sewage inputs and without substantial upland clean water supplies to dilute them.

Sixty-two sites have been sampled every two months between May 2002 and May 2003. The sites are a mixture of main river locations, as well as downstream samples of major sub-catchments, and mid catchment samples at points of changing land use or anthropogenic impact. Figure 1 shows the location of the sample sites. We measured a range of spectrophotometric (both absorbance and fluorescence) parameters in river water at the sixty-four sample sites under a range of flow regimes from summer base flow (August 2002; ~30 m³ s⁻¹ at site 30) through to winter storm flow (November 2002; ~200 m³ s⁻¹) and during winter low flows during extensive snow cover (January 2003; ~50 m³ s⁻¹). Water samples were collected in 30 ml polypropylene bottles which had been cleaned in 10% HCl and distilled water. Samples were kept refrigerated, and upon return from the field were filtered (Whatman GF/C ashed glass microfibre filter papers),
before being analysed within 48 hrs. Such a delay between sampling, filtering and then analysis was unavoidable given the time taken (2 days of fieldwork) to sample a catchment of this size. Some changes in fluorescence during storage due to this delay must be anticipated, especially for more labile samples (Baker, 2002b). Fluorescence measurements were undertaken using a Perkin-Elmer LS-50B luminescence spectrometer as described elsewhere (Baker, 2001). The Raman intensity (excitation 348 nm, emission $\sim$ 396 nm, 5 nm slit width) of distilled water in sealed water cell was used as a standard. This permitted testing for machine stability, and also provides a means of inter-laboratory comparison. All data presented here is calibrated to a Raman peak intensity of $20.0$ units at $396$ nm emission wavelength. Absorption at $254$ nm, $340$ nm and $410$ nm was undertaken using a WPA Lightwave UV–VIS spectrometer, to provide a check for inner-filtering effects. The latter are particularly observed in waters of high concentrations of dissolved natural organic matter that are often highly absorbent in ultraviolet light. In these conditions, emitted fluorescence is often reabsorbed by dissolved organic matter within the sample cuvette, resulting in a quenching of emitted fluorescence and a resultant decrease in intensity; Ohno, 2002). We ran serial dilutions on a subset of samples, and observed that samples from the peat dominated the North Tyne catchment, which were visibly coloured, often exhibited inner-filtering, with absorbance maxima of $>0.3$ cm$^{-1}$ at $254$ and $340$ nm and a decreased fulvic-like fluorescence intensity of $>10\%$. However, one of the advantages of fluorescence analysis is the rapid analysis time, an advantage that is negated if samples have to be corrected for inner-filtering. Hence no inner-filtering correction was applied to the dataset and raw fluorescence values were used as we wished to test if the raw fluorescence data could be used as a potential water quality determinant.

Our sample sites are also those used by the Environment Agency in their general water quality assessment scheme. The General Quality Assessment scheme (GQA) is the

![Image](https://iwaponline.com/wst/article-pdf/52/9/199/434515/199.pdf)
national method for classifying water quality in rivers and canals. The scheme provides a way of comparing river quality from one river to another and for looking at changes through time: this assessment includes chemical and nutrient analyses including orthophosphate, nitrate, dissolved oxygen, ammonia and biochemical oxygen demand. Ammonia, biochemical oxygen demand (BOD), and dissolved oxygen are used as measurements of organic pollution. Phosphate and nitrate are used to indicate possible existing or future problems of eutrophication: additionally nitrate is useful where river water may be abstracted for drinking water and needs to comply with the EC Drinking Water and Nitrate Directives. GQA analyses are on samples from routine, pre-planned sampling programmes with samples analysed by accredited laboratories: to avoid bias all extra data collected for special surveys or in response to incidents or accidents are ignored. All data and results for all rivers are made available to the public. Standard analytical methods are used (Standing Committee of Analysts Methods for the Examination of Waters and Associated Materials, 1980, 1981a,b, 1988). Monthly samples that were taken for the GQA assessment over the same period as the fluorescence sampling have been used here. Comparison between GQA and fluorescence results is not on paired samples, chemical water quality parameters within the Environment Agency sample collection program are sampled on different tributaries on different days, and fluorescence sampling occurred over two days that rarely overlapped with Environment Agency sampling dates. Environment Agency samples (every 4 weeks) were also taken more frequently than fluorescence samples (every 8 weeks). Such sampling methods permit a statistical analysis of the relationship between fluorescence intensity and chemical water quality parameters, based on the mean and standard deviation of each parameter at each sample site. Such an approach is similar to that used by the Environment Agency to determine river water quality standards and objectives.

In order to compare laboratory bench and newly developed portable field based fluorescence spectrometers, 125 sub samples of river water were taken for analysis using the portable SMF2 spectrophotometer (SafeTrainingSystems Ltd, Wokingham, UK). The SMF2 has a xenon flash lamp as excitation source, with the flash focused through a band pass filter to select the required excitation wavelength. For tryptophan, a combination of three interference filters was used with a peak excitation wavelength of 280 nm and a full-width half-maximum of 60 nm. No cut off filter was used as the 280 excitation peak was well separated from the 350–360 nm measuring area for the fluorescence signal. The SMF2 has a spectral display on the instrument that allows the operator to observe the peak shape of the tryptophan-like fluorescence centre and to observe any other unexpected fluorescence which could affect the quantification of the tryptophan-like fluorescence. The instrument also has incorporated a 9 V rechargeable battery which allows 4–8 hours of operation. A standard 1 cm quartz cuvette was used for the water samples and sets of 3 repeat analyses made for each sample. Calibration samples were run before the start of any analysis of water samples by analyzing distilled water. For these samples the intensity measured was greater than zero as it includes a contribution from Raman emission within the range of the filter sets used. Analyses were adjusted to a constant intensity of 22 units, and this value subtracted from subsequent water analyses.

Results
Environment Agency chemical water quality data demonstrate that for the majority of rural sample sites the chemical water quality is very good, with dissolved oxygen ~100%, BOD <1 mg/l and ammonia <0.1 mg/l. A few sites on urban tributaries have much poorer water quality. For example, sites 1–3 are on the Ouseburn which is known to be impacted by sewerage failures: additionally during the study period sites 1 and 2,
downstream of Newcastle International Airport, were affected by a >60 mg/l BOD event in January 2003 due to propylene glycol de-icer runoff from the airport. Sites 34 and 35 are on the River Don, which also suffers from sewerage inputs from combined sewer overflows, and sites 36–39 are on the River Team which comprises treated sewage as a significant proportion of flow. In addition, site 36 is downstream of a pumped mine water discharge and a sewage treatment works, the combination of which can provide a substantial proportion of total river discharge, as well as a tributary that suffers from leachate from an unlined landfill. The combination of these inputs explains the high ammonia concentration at site 36.

Comparing the correlation between fluorescence and chemical water quality determinants shows that there are statistically significant relationships between nitrate, phosphate and ammonia and tryptophan-like fluorescence (at either 220 nm and/or 280 nm excitation centres) (Table 1). This suggests that the relationship between protein-like fluorescence and potential pollutants such as treated and untreated sewage and farm wastes is reflected at a catchment-wide scale. However, three sites within the dataset have statistically outlying data, from non-fluorescent propylene glycol de-icer (sites 1 and 2) and landfill leachate pollution (site 36). Therefore all outlying data were removed from the dataset: the January 2003 BOD from sites 1 and 2 and all ammonia data from site 36, and the correlations recalculated. In this case, the strength of the correlation between tryptophan-like fluorescence and the chemical water quality determinants increased significantly, with tryptophan-like fluorescence becoming the most significant explanatory variable in every case. Tryptophan-like fluorescence intensity at the 280 nm excitation/350 nm emission fluorescence centre correlates with both phosphate ($r = 0.80$) and nitrate ($r = 0.87$), whereas tryptophan-like fluorescence intensity at the 220 nm excitation/350 nm emission wavelength centre correlates with BOD ($r = 0.85$), ammonia ($r = 0.70$) and dissolved oxygen ($r = -0.65$). The weakest correlations are observed between tryptophan-like fluorescence intensity and ammonia concentration and dissolved oxygen. For the former, the weaker correlation is due to good ammonia treatment within the wastewater treatment plants within the catchment, such that ammonia is stripped, yet a residual tryptophan-like fluorescence signature from the wastewater DOM remains: essentially ammonia concentration is not a water quality issue in the Tyne catchment. Despite this, the highest values of tryptophan-like fluorescence and ammonia are found at the urban catchment sample sites on the rivers Don, Team and Ouseburn. Finally, the weak correlation between dissolved oxygen and tryptophan-like fluorescence reflects the aeration of the river as described earlier, such that natural aeration limits any water quality impacts on dissolved oxygen. Only one site (site 36) has depressed oxygen levels, due to the impacts of landfill leachate and treated wastewater that discharge into the river Team just upstream of this sample site.

We also performed stepwise regression to investigate if the analysis of the fluorescence intensities and wavelengths of all possible fluorescence centres adds further statistical strength to the observed correlations between tryptophan-like fluorescence intensity and the chemical water quality determinants. This is also shown in Table 1, and shows that although the addition of one or two more fluorescence parameters does increase the correlation, the improvement in explanatory power is negligible compared to the initial tryptophan-like fluorescence – chemical water quality relationship. We also repeated all the stepwise regressions including absorbance as a determining variable; absorbance was observed to not be a statistically significant determinant for any of the chemical water quality parameters.

Correlations between fluorescence measured by the portable spectrophotometer with a conventional bench machine was 0.87 ($n = 125$), demonstrating that the portable
Table 1 Regression equations between fluorescence and chemical water quality parameters. $H_I$ is the intensity of the humic-like fluorescence centre; $F_{ex}$, $F_{em}$ and $F_I$ are the excitation and emission wavelengths and intensity of the fulvic-like fluorescence centre, and $T_{280}$, $T_{220}$ and Tyro are intensities of the tryptophan-like and tyrosine-like fluorescence centres. Correlation coefficients are shown for both simple linear regression with one correlant, and for the stepwise regression equation shown.

<table>
<thead>
<tr>
<th>Chemical water quality determinand</th>
<th>Stepwise regression</th>
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<th>Single parameter r</th>
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</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>$-8.55 + 0.01808 T_{280} + 0.02536 F_{ex} - 0.002082 H_I$</td>
<td>0.894</td>
<td>0.799</td>
</tr>
<tr>
<td>Nitrate</td>
<td>$-0.006798 + 0.112 T_{280} - 0.07127 Tyro$</td>
<td>0.935</td>
<td>0.874</td>
</tr>
<tr>
<td>BOD (all data)</td>
<td>$-1.586 + 0.0569 Tyro + 0.01269 F_{em}$</td>
<td>0.754</td>
<td>0.675</td>
</tr>
<tr>
<td>BOD (excluding sites 1,2)</td>
<td>$-13.467 + 0.01578 T_{220} + 0.03232 F_{em}$</td>
<td>0.903</td>
<td>0.846</td>
</tr>
<tr>
<td>DO</td>
<td>$102.578 - 0.07328 T_{220}$</td>
<td>0.646</td>
<td>0.646</td>
</tr>
<tr>
<td>Ammonia (all data)</td>
<td>$-0.224 + 0.002733 H_I - 0.002171 F_I$</td>
<td>0.845</td>
<td>0.784</td>
</tr>
<tr>
<td>Ammonia (excluding site 36)</td>
<td>$-0.04166 + 0.0016 T_{220}$</td>
<td>0.703</td>
<td>0.703</td>
</tr>
</tbody>
</table>
spectrophotometer does correlate with tryptophan-like fluorescence intensity measured using the bench spectrophotometer (Figure 2). Replicate samples demonstrated that the SMF2 fluorescence intensity is reproducible to ±3 units: for low fluorescence intensity, good quality river waters this amounts to ~20% error, whereas for urban rivers and wastewaters it is a ~5% error. Samples with fluorescence intensity of >100 on the Cary Eclipse and >60 on the SMF-2 are all from rivers that have water quality issues (defined as failing their chemical water quality targets as defined by the Environment Agency of England and Wales).

We investigated the relationship between the mean and standard deviation tryptophan-like fluorescence intensity and the GQA for each site for the year 2002. The GQA is scored from A to F, where A is highest water quality and F the lowest, and is based on the dissolved oxygen, BOD and ammonia. Results are presented in Figure 1, and demonstrate a strong relationship between the GQA grade and mean tryptophan-like fluorescence intensity (at the 220 nm excitation/350 nm emission centre), although the small number of poor water quality sites limits the number of sites scored at grades D and E. Figure 3 suggests that with a larger dataset that includes a greater proportion of poor quality waters, water quality standards could be determined and assessed using tryptophan-like fluorescence as a chemical water quality determinant.

Conclusions
In the Tyne catchment, with the exception of the airport de-icer and landfill leachate impacted sample sites, the chemical water quality determinants are predominantly detecting sewerage derived DOM from combined sewer overflows, cross connected sewers and wastewater treatment works discharges into rivers where the discharge provides a significant proportion of total river flow. Therefore, the strong correlations between BOD, nitrate and phosphate and tryptophan-like fluorescence intensity suggests that tryptophan-like fluorescence can be used as a proxy for these parameters where sewerage sources of DOM are important. The findings demonstrate that upscaling of the tryptophan-like
fluorescence intensity—water quality relationship observed at the smaller scales of small urban catchment (Ouseburn, Baker, 2002a; Baker et al., 2003) and downstream of treated wastewater outfalls (Baker, 2001) to that of a large catchment is possible. The rapid analysis time required to produce a fluorescence EEM (less than 1 minute) also permits the real-time analysis of waters. Additionally, we demonstrate that portable spectrophotometric detection of tryptophan-like fluorescence in river waters is now possible. Portability has widespread applications in the water industry. Tryptophan-like fluorescence is widely associated with pollution from human and animal wastes: a field based spectrophotometer permits the in situ sourcing of pollution and rapid remediation.

References


Figure 3 Comparison of tryptophan-like fluorescence intensity and Environment Agency General Water Quality Assessment, 2002


