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Protein-like fluorescence intensity as a possible tool for determining river water quality

Andy Baker[1]* and Roger Inverarity[2]

[1] *School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*
[2] *Environment Agency, Tyneside House, Skinnerburn Road, Newcastle NE4 7AR, UK*

Abstract:

The results of a comparison between chemical water quality determinants and river water fluorescence on the River Tyne, NE England, demonstrate that tryptophan-like fluorescence intensity shows statistically significant relationships between nitrate, phosphate, ammonia, biochemical oxygen demand (BOD) and dissolved oxygen. Tryptophan-like fluorescence intensity at the 280 nm excitation/350 nm emission wavelength fluorescence centre correlates with both phosphate ($r = 0{\cdot}80$) and nitrate ($r = 0{\cdot}87$), whereas tryptophan-like fluorescence intensity at the 220 nm excitation/350 nm emission wavelength centre correlates with BOD ($r = 0{\cdot}85$), ammonia ($r = 0{\cdot}70$) and dissolved oxygen ($r = -0{\cdot}65$). The strongest correlations are between tryptophan-like fluorescence 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 removal of the ammonia signal by wastewater treatment, and that with dissolved oxygen due to the natural aeration of the river such that this is not a good indicator of water quality. The observed correlations only hold true when treated sewage, sewerage overflows or cross connections, or agricultural organic pollutants dominate the water quality—this is not true for two sites where airport deicer (propylene glycol, which is non-fluorescent) or landfill leachate (which contains high concentrations of humic and fulvic-like fluorescent DOM) dominate the dissolved organic matter in the river. Mean annual tryptophan-like fluorescence intensity agrees well with the General Water Quality Assessment as determined by the England and Wales environmental regulators, the Environment Agency. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS chemical water quality; fluorescence; River Tyne; tryptophan; ammonia; biochemical oxygen demand; dissolved oxygen

INTRODUCTION

Dissolved organic matter (DOM) has distinctive spectrophotometric properties in terms of both absorption and fluorescence. As well as strong absorption in the ultraviolet, 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 such as humic and fulvic-like material, as well as fluorescent protein-like fluorophores (for a review of possible fluorescence centres see Coble, 1996 and Stedmon *et al*., 2003 and for typical EEMs see Baker, 2001). Studies of FDOM EEM properties have principally focused on wastewater characterization within the treatment process (for example see Reynolds and Ahmad, 1997; Westerhoff *et al*., 2001; Vasel and

* Correspondence to: Dr Andy Baker, School of Geography, Earth & Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

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Praet, 2002) as well as DOM characterization in marine and estuarine waters (for example see Coble *et al*., 1990; Mopper and Schultz, 1993; Mayer *et al*., 1999; Parlanti *et al*., 2001), but more recently research has included riverine DOM. For example, Stedmon *et al*. (2003) use fluorescence to derive five DOM fractions in a Danish freshwater and estuarine catchment. Fluorescence has been demonstrated to be able to detect the differences between both anthropogenic and natural DOM sources in rivers impacted by sewerage effluents (Baker, 2001; Baker *et al*., 2003). Anthropogenic DOM sources such as farm wastes, sewage treatment outfall or sewerage overflows are all characterized by high levels of protein-like (tryptophan-like and/or tyrosine-like) fluorescence (Baker, 2001, 2002b). Baker (2002a) shows how a combination of optical properties (fluorescence and absorbance) together with conventional total organic carbon measurements can be used to discriminate both temporal and spatial variations of DOM in a small urban catchment. Fluorescence can also be used to trace DOM within 'natural' catchments: McKnight *et al*. (2001, 2003) used fulvic-like fluorescence wavelength variations as a tracer of microbially vs terrestrially derived fulvic material in an alpine/sub-alpine catchment in the USA; Thoss *et al*. (2000) used fluorescence to trace DOM fractions in six catchments of contrasting land use in North Wales; Newson *et al*. (2001) and Bolton (2004) have also used fluorescence properties of coloured river water as a natural tracer in a small peaty subcatchment of the River Eden (Coalburn).

Previous research has demonstrated that the measurement of FDOM EEMs in micro (<40 km^2) scale catchments (both urban and rural) can provide useful information on DOM sources (Newson *et al*., 2001; Baker, 2002b). Protein-like fluorescence centres observed in EEMs are described 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 fluorescence 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). These locations in optical space are where tryptophan and tyrosine laboratory standards fluoresce; however, it is not known whether tryptophan or tyrosine *per se* are present as DOM, or rather similarly structured groups within DOM that have similar fluorescence properties (Reynolds, 2003). Although it is not known how these fluorescence centres relate to the structure of riverine DOM, their presence in rivers with anthropogenic DOM inputs requires further investigation. In particular, to determine if the relationship between increased protein-like fluorescence (tryptophan-like and tyrosine-like) intensity and anthropogenic DOM inputs is maintained in a larger scale (>1000 km^2) 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. In addition, it is useful to 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) that are used to determine river water quality in England and Wales. Although some of these chemical determinants do provide information as to a possible source of input (for example, phosphates are often predominantly derived from sewage effluent), many do not (biochemical oxygen demand provides a general index of oxygen demand that is time-consuming to perform). In contrast, fluorescence can be measured rapidly, portable spectrophotometers permit field-based EEM analysis (Hart and Jiji, 2002) and the simultaneous determination of several fluorescence centres using EEMs could in a single analysis provide several correlations between fluorescence and chemical water quality. Therefore we present the results of a comparison between standard chemical water quality determinants (as performed by the England and Wales water quality regulator, the Environment Agency) and fluorescence on water samples from the Tyne catchment in NE England.

METHODOLOGY

The River Tyne has a catchment area of 2935 km^2 and comprises two main tributaries, the North and South Tyne which meet near Hexham (sample site 25, see Figure 1). The North Tyne rises in the Cheviot Hills near the Scottish Border, the South Tyne in the Cumbrian Pennines. The other main tributaries of the Tyne are the River Rede and Tarset Burn on the North Tyne, rivers Allen and Nent on the South Tyne, and the River

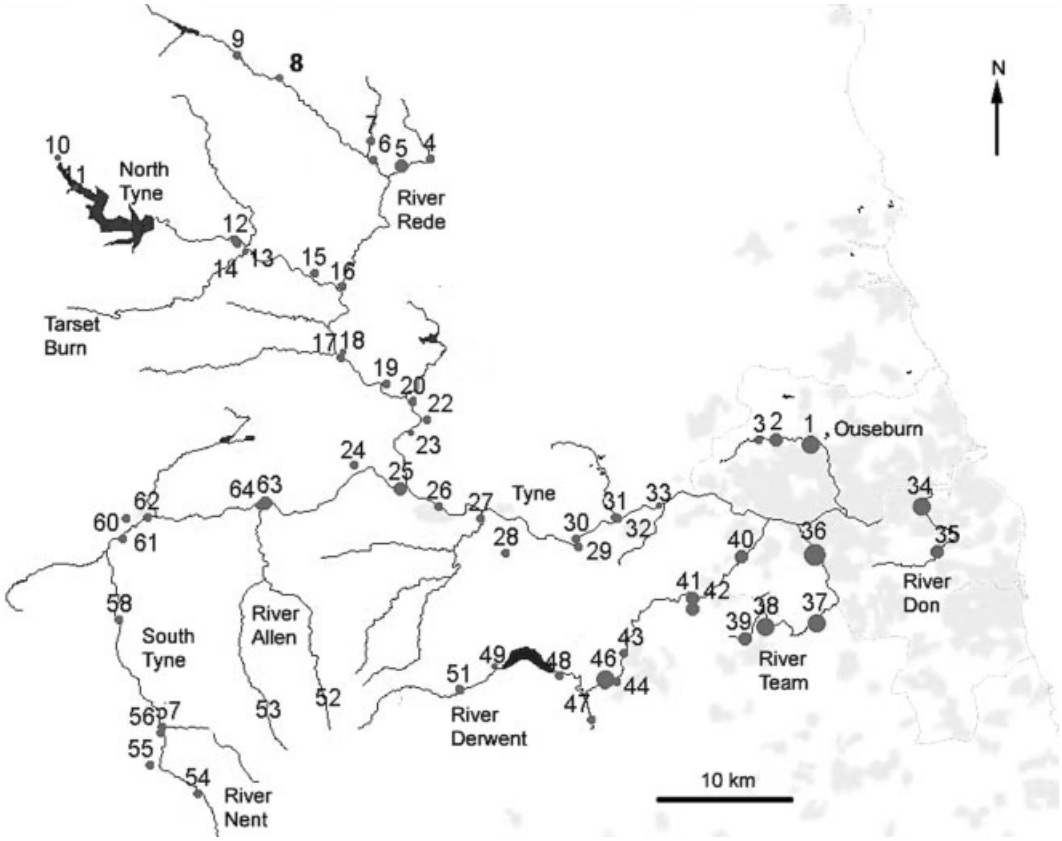

Figure 1. The Tyne catchment, NE England, showing location of the sample sites. Sizes of proportional circles reflect tryptophan-like fluorescence intensity as measured in the January 2003 sample run. Urban areas are shown in light grey, and the city limit for Newcastle-upon-Tyne by the grey line

Derwent, River Team, Ouseburn and River Don which enter the Tyne in its tidal section (downstream of site 33, Figure 1). Land-use and its relationship with water quality on the Tyne was a focus of a major study (NELUP: North East Land Use Project); recent catchment land use is therefore well understood (Adams *et al*., 1995; Wadsworth and O'Callaghan, 1995; Dunn *et al*., 1996; Lunn *et al*., 1996). 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 [River Don (sites 34, 35); Ouseburn (sites 1–3); River Team (sites 36–39); and the lower reaches of the River Derwent (sites 40–42)] 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 as part of a larger project to investigate the spectrophotometric variations in river water in the catchment and its relation to land-use (results are to be published elsewhere). The sites are a mixture of main river locations, as well as downstream samples of major subcatchments, 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 64 sample sites under a range of flow regimes from summer base flow (August 2002; $\sim$30 m^3 s^{-1} at site 30) through to winter storm flow (November 2002; $\sim$200 m^3 s^{-1}) and during winter low flows during extensive snow cover (January 2003; $\sim$50 m^3 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 h. Such a delay between sampling, filtering and then analysis was unavoidable given the time taken (two 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 a sealed water cell was used as 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 $\sim$396 nm emission wavelength. Absorption at 254 nm, 340 nm and 410 nm was undertaken using a WPA lightwave UV–VIS spectrometer, both to investigate the relationship between this spectrophotometric technique and land-use (results not considered further in this paper), as well as 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 (Mobed *et al*., 1996; Ohno, 2002). We ran serial dilutions on a subset of samples, and observed that samples from the peat-dominated 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 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 programme 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 four weeks) were also taken more frequently than fluorescence samples (every eight 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.

RESULTS AND DISCUSSION

Table I presents the summary of all results for the 62 sample sites. Environment Agency chemical water quality data demonstrate that for the majority of sample sites the chemical water quality is very good, with dissolved oxygen ~100%, BOD <1 mg l^{-1} and ammonia <0·1 mg l^{-1}. A few sites on urban tributaries have much poorer water quality. For example, sites 1–3 are on the Ouseburn (and correspond to sites 16, 10 and 3 respectively of Baker, 2002a), 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^{-1} BOD event in January 2003 due to propylene glycol deicer runoff from the airport (Turnball and Bevan, 1995 provide details of airport-derived pollution on the river from urea applications in the 1980s and 1990s). 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 (sites 36–38 are downstream of the East Tanfield wastewater treatment works, whose impact on river water fluorescence was investigated by Baker, 2001). 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.

Absorbance data for the sample sites show a strong variation between the North Tyne, which is predominantly an upland peat catchment, and the South Tyne, whose source is in limestone uplands with brown earth and thin peats. Samples from the peaty catchments of the rivers North Tyne and Rede have absorbance high enough to be affected by inner-filtering (Ohno, 2002); hence fluorescence intensities might be expected to be decreased at these locations. Fluorescence results are reported as both excitation and emission wavelengths and intensity of the observed peaks. Those often defined as 'humic-like' and 'fulvic-like' (although their precise nature is poorly understood) are located at 220–250 nm excitation and 400–460 nm emission ('humic-like'), and at 300–350 nm excitation and 400–460 nm emission ('fulvic-like'). Increases in wavelength of both excitation and emission of the fulvic-like peak can be due to increasing molecular weight, increasing aromacity or increasing inner-filtering effects (Ohno, 2002; Bolton, 2004). In our case, without inner-filtering correction applied to our dataset, the latter is the most dominant effect, with highest excitation and emission wavelengths correlating with high absorbance at sites 12, 13 and 14. For the protein-like fluorescence centres attributed to tryptophan-like and tyrosine-like fluorescence, only fluorescence intensities are reported as significant wavelength variations did not occur. Protein-like fluorescence intensities can be seen to be highest in the urban catchments of the Ouseburn, Team and Don, and this is also shown by proportional circle size in Figure 1.

Table II presents the correlation [Pearson rank correlation due to the presence of statistically outlying data at sites 1, 2 (biochemical oxygen demand) and 36 (ammonia)] between the mean annual concentration or intensity of each of the variables between the 62 sample sites. Within the Environment Agency dataset, phosphate, ammonia and nitrate have the strongest correlation, suggesting a similar source for all three. Nitrate is often agriculturally derived: however, although the Tyne contains a large proportion of agricultural land-use, almost all of this is extensive in nature, with only a small area (predominantly around the Whittle Burn upstream of site 31) that can be considered intensive. Therefore the correlation between nitrate and ammonia and phosphate, the latter two being indicators of sewage pollution, confirms a predominant sewage source of nitrate in the Tyne. Weaker correlations occur with BOD and dissolved oxygen. For the former, it is due at least in part to the influence of the two deicer pollution events at sites 1 and 2, which had high BOD but no nitrate, phosphate or ammonia. For dissolved oxygen, it is due to the geomorphology of the Tyne catchment in general: the river is typically well aerated with a combination of steep gradients on tributaries flowing in post-glacially incised valleys, as well as regular ripple-pool sequences, chutes and rapids in the main river. For the catchment as a whole, dissolved oxygen is therefore not a good measure of water quality.

Within the spectrophotometric data, absorbances at 254, 340 and 410 nm correlate strongly with each other, reflecting the nature of DOM absorbance with little structure and decreasing absorbance at increasing

Table I. Summary spectrophotometric (absorbance; humic-like, fulvic-like, tryptophan-like and tyrosine-like fluorescence) and chemical water quality data. Mean and 1σ standard deviation are for ~12 samples for the Environment Agency (EA) chemical water quality data, and six samples for the spectrophotometric data

D	Site name		Chemical-water-quality parameters					Spectrophotometric parameters												
			O-Phosphate (EA data) (mg l^{-1})	Nitrate (EA data) (mg l^{-1})	BCD (EA data) (mg l^{-1})	DO (EA data) (%)	Ammonia (EA data) (mg l^{-1})	Absorbance at			Humic-like fluorescence			Fulvic-like fluorescence			Tryptophan at 250 mm intensity	Tyrosine intensity	Tryptophan at 220 mm intensity
								254 nm	340 nm	410 nm	Excitation (nm)	Emission (nm)	Intensity	Excitation (nm)	Emission (nm)	Intensity			
1	OUSE BURN AT GOSFORTH	mean	0·210	2·087	6·891	100	0·300	0·190	0·056	0·020	238	413	315	327	417	180	78	76	170
		stdev	0·088	1·358	16·106	35	0·300	0·108	0·041	0·014	4	6	71	11	4	44	14	44	71
2	OUSE BURN AT BRUNTON	mean	0·144	2·546	9·435	86	0·096	0·187	0·053	0·019	236	418	338	329	419	187	79	77	177
		stdev	0·060	1·178	28·155	19	0·126	0·108	0·041	0·015	1	9	88	8	8	52	26	51	89
3	OUSE BURN AT WOOLSINGTON	mean	0·193	2·875	1·921	78	0·195	0·160	0·046	0·019	237	412	265	331	418	140	57	45	119
		stdev	0·081	1·747	0·778	16	0·298	0·130	0·049	0·022	4	8	44	10	3	46	8	14	44
4	ELSDON BURN AT ELSDON	mean	0·030	0·532	1·578	98	0·035	0·347	0·121	0·043	235	432	242	338	436	143	33	41	75
		stdev	0·014	0·247	0·438	6	0·008	0·292	0·108	0·036	10	11	42	5	12	60	17	32	53
5	ELSDON BURN AT A696	mean	0·024	0·495	1·694	96	0·032	0·338	0·116	0·041	235	426	250	339	438	147	32	33	73
		stdev	0·010	0·294	0·652	4	0·005	0·286	0·104	0·036	1	10	38	3	8	58	10	11	30
6	REDE AT OTTERBURN	mean	0·045	0·488	1·512	93	0·097	0·711	0·260	0·092	236	435	204	339	446	151	21	21	45
		stdev	0·049	0·501	0·473	10	0·230	0·506	0·198	0·074	1	7	50	2	9	45	7	11	23
7	OTTER BURN AT OTTERBURN	mean	0·058	0·495	1·848	94	0·064	0·575	0·205	0·069	239	441	256	341	447	180	32	29	64
		stdev	0·046	0·420	0·632	8	0·043	0·337	0·127	0·043	10	8	50	3	11	55	14	23	30
8	SILLS BURN AT ASS ROAD	mean	0·036	0·192	1·748	97	0·034	0·740	0·272	0·096	243	443	223	347	444	169	21	15	40
		stdev	0·022	0·003	0·555	6	0·008	0·478	0·188	0·071	13	5	82	19	10	59	8	8	20
9	REDE AT COTTON SHOPEFOOT	mean	0·027	0·348	1·769	98	0·035	0·412	0·149	0·055	234	439	196	339	446	128	21	22	50
		stdev	0·010	0·235	0·744	7	0·013	0·201	0·075	0·029	2	8	27	2	6	35	4	8	14
10	NORTH TYNE US KIELDER	mean	0·029	0·238	1·543	100	0·031	0·564	0·223	0·084	238	443	142	349	449	103	14	24	34
		stdev	0·021	0·148	0·363	3	0·002	0·483	0·189	0·073	5	9	40	18	11	36	4	14	17
11	LEWISBURN US KIELDER	mean	0·028	0·200	1·529	101	0·034	0·747	0·272	0·097	239	440	167	341	448	136	16	14	35
		stdev	0·009	0·037	0·329	4	0·009	0·064	0·022	0·011	10	11	23	2	7	15	3	8	5
12	NORTH TYNE AT TARSET	mean	0·025	0·329	1·581	100	0·031	0·760	0·280	0·100	236	438	170	338	449	141	17	17	35
		stdev	0·009	0·083	0·650	6	0·003	0·151	0·059	0·022	2	8	24	3	4	27	2	11	8
13	CHIRDON BURN AT TARSET	mean	0·031	0·222	1·804	98	0·034	1·079	0·397	0·141	240	443	157	342	451	146	13	11	24
		stdev	0·010	0·780	0·599	4	0·009	0·515	0·187	0·062	8	6	39	4	6	30	5	8	17
14	TARSET BURN AT TARSET	mean	0·030	0·216	1·865	98	0·038	1·012	0·374	0·130	241	445	161	340	447	157	16	14	30
		stdev	0·090	0·048	0·905	4	0·016	0·607	0·232	0·080	9	9	52	3	4	42	5	11	17
15	HARESHAW BURN AT BELLINGHAM	mean	0·026	0·234	1·588	95	0·032	0·667	0·248	0·089	242	442	216	348	451	162	21	25	45
		stdev	0·008	0·069	0·473	6	0·009	0·479	0·183	0·066	10	11	57	19	10	48	7	14	21
16	REDE AT REDESMOUTH	mean	0·036	0·311	1·973	99	0·033	0·541	0·196	0·073	236	432	246	339	447	164	27	29	59
		stdev	0·016	0·135	0·631	7	0·005	0·245	0·093	0·039	2	6	42	3	2	48	6	15	17
17	WARKS BURN AT WARK	mean	0·160	0·302	1·850	99	0·032	0·968	0·357	0·128	236	439	186	348	451	166	18	14	35
		stdev	0·436	0·108	0·649	4	0·003	0·570	0·223	0·079	3	7	66	18	11	42	8	11	22
18	NORTH TYNE AT WARK	mean	0·029	0·291	1·756	99	0·031	0·783	0·284	0·102	240	440	179	340	449	148	18	15	37
		stdev	0·008	0·091	0·507	3	0·002	0·340	0·115	0·041	8	6	51	4	8	35	5	5	10

19	GUNNERTON BURN AT BURNMOUTH	mean	0·070	1·836	1·400	99	0·039	0·215	0·068	0·027	238	429	240	337	427	142	37	29	67
		stdev	0·027	0·525	0·394	3	0·019	0·180	0·054	0·023	7	11	62	4	8	71	7	10	17
20	SWINBURN AT BARRASFORD	mean	0·029	2·126	1·611	101	9·038	0·236	0·065	0·025	235	426	270	329	430	144	40	29	70
		stdev	0·008	0·924	1·114	3	0·030	0·080	0·012	0·008	1	7	44	10	8	27	11	8	23
21	NORTH TYNE AT BARRASFORD	mean	0·029	0·529	1·568	100	0·036	0·682	0·249	0·092	233	435	192	339	449	151	20	21	37
		stdev	0·008	0·254	0·425	3	0·014	0·200	0·073	0·026	6	7	31	3	8	37	3	15	9
22	ERRING BURN AT CHOLLERTON	mean	0·030	2·612	1·635	97	0·031	0·207	0·059	0·023	235	431	229	331	428	121	34	28	60
		stdev	0·008	1·206	0·421	6	0·004	0·053	0·012	0·006	1	6	27	10	5	23	6	10	15
23	NORTH TYNE AT CHOLLERFORD	mean	0·026	0·543	1·777	92	0·041	0·789	0·290	0·106	240	442	183	338	449	150	19	18	37
		stdev	0·009	0·270	0·678	6	0·020	0·392	0·148	0·053	8	9	45	3	6	40	5	8	17
24	NEWBROUGH BURN AT NEWBROUGH	mean	0·035	1·320	1·723	97	0·040	0·161	0·047	0·017	234	427	191	337	431	96	27	29	54
		stdev	0·022	0·503	0·370	4	0·028	0·169	0·062	0·019	3	7	59	4	8	52	6	11	15
25	SOUTH TYNE AT WARDEN BRIDGE	mean	0·054	0·646	1·855	100	0·058	0·404	0·158	0·058	235	436	211	339	445	133	30	35	57
		stdev	0·067	0·328	0·423	7	0·067	0·222	0·089	0·034	1	9	41	3	8	42	5	15	12
26	TYNE AT HEXHAM	mean	0·028	0·629	1·564	95	0·032	0·624	0·231	0·086	237	442	196	338	443	143	23	22	46
		stdev	0·008	0·328	0·427	6	0·005	0·279	0·108	0·039	6	10	33	2	3	34	6	10	15
27	DEVILS WATER AT DILSTON HALL	mean	0·024	0·996	1·479	102	0·034	0·304	0·100	0·037	229	425	220	336	437	121	30	35	70
		stdev	0·006	0·601	0·329	4	0·011	0·295	0·105	0·033	7	9	20	6	7	38	7	17	26
28	MARCH BURN AT DIPTON HOUSE	mean	0·187	2·889	1·582	97	0·044	0·223	0·070	0·028	235	430	231	336	429	133	36	31	67
		stdev	0·115	0·589	0·375	7	0·016	0·155	0·055	0·017	0	8	34	7	6	45	6	13	21
29	STOCKSFIELD BURN AT STOCKSFIELD	mean	0·041	2·418	1·511	97	0·036	0·173	0·051	0·019	234	426	220	335	427	119	37	33	70
		stdev	0·017	1·269	0·361	8	0·008	0·145	0·052	0·016	2	8	35	4	9	44	5	13	17
30	TYNE AT BYWELL	mean	0·039	0·876	1·514	97	0·041	0·638	0·231	0·084	238	439	197	339	446	148	22	23	45
		stdev	0·014	0·307	0·445	3	0·012	0·277	0·108	0·041	7	10	32	4	7	40	6	11	14
31	WHITTLE BURN AT OVINGHAM	mean	0·053	3·913	1·442	99	0·037	0·223	0·071	0·026	235	422	233	335	428	128	42	34	77
		stdev	0·040	2·823	0·406	4	0·013	0·143	0·060	0·023	0	5	48	6	8	43	8	27	27
32	TYNE AT OVINGHAM	mean	0·038	0·758	1·496	96	0·045	0·649	0·234	0·085	236	435	198	339	443	149	23	20	48
		stdev	0·014	0·295	0·377	7	0·018	0·279	0·106	0·038	10	14	31	2	6	43	7	11	17
33	TYNE AT WYLAM BRIDGE	mean	0·046	0·964	1·708	96	0·115	0·638	0·198	0·084	238	443	225	340	445	167	23	21	45
		stdev	0·020	0·307	0·425	6	0·093	0·252	0·133	0·038	7	10	41	1	7	33	6	12	16
34	DON AT JARROW CEMETERY	mean	0·332	2·794	3·036	80	0·567	0·173	0·048	0·021	238	412	308	328	420	151	90	83	196
		stdev	0·186	1·439	1·966	15	0·896	0·093	0·032	0·015	4	8	77	13	6	54	16	45	81
35	DON AT MOUNT PLEASANT	mean	0·159	2·806	2·536	90	0·397	0·131	0·035	0·017	235	414	220	337	421	109	52	55	114
		stdev	0·117	1·408	2·294	22	0·547	0·101	0·037	0·017	1	5	66	5	8	53	3	23	28
36	TEAM AT THIRD AVENUE BRIDGE	mean	0·725	5·202	5·230	72	1·405	0·115	0·032	0·017	238	413	605	331	416	138	108	72	285
		stdev	0·442	1·818	3·220	15	1·087	0·056	0·018	0·009	4	5	763	9	1	43	19	15	32
37	TEAM US SEDGEHILL	mean	1·351	8·528	2·700	97	0·310	0·162	0·036	0·017	241	415	316	334	419	179	96	51	197
		stdev	0·642	2·802	2·003	8	0·424	0·033	0·007	0·006	5	4	54	5	2	38	29	10	60
38	TEAM AT BEAMISH BRIDGE	mean	1·844	10·374	4·092	89	0·068	0·167	0·064	0·023	242	419	302	337	421	199	135	64	228
		stdev	0·954	3·529	2·789	9	0·573	0·026	0·045	0·005	5	6	48	4	3	50	52	15	71
39	TEAM AT TANTOBIE ROAD	mean	0·052	1·253	1·750	95	0·139	0·117	0·028	0·014	235	415	252	333	421	116	52	63	120
		stdev	0·035	0·558	0·657	6	0·333	0·039	0·011	0·006	1	8	47	6	4	27	6	13	25
40	DERWENT AT CLOCKBURN DRIFT	mean	0·420	3·263	2·682	101	0·074	0·151	0·045	0·020	235	427	212	335	428	105	53	49	106
		stdev	0·130	0·055	2·847	8	0·028	0·051	0·018	0·010	0	9	28	7	7	21	8	13	21

(*continued overleaf*)

Table I. (*Continued*)

D	Site name		Chemical-water-quality parameters					Spectrophotometric parameters											
								Absorbance at			Humic-like fluorescence			Fulvic-like fluorescence			Trypto-phan at 250 mm intensity	Tyro-sine intensity	Trypto-phan at 220 mm intensity
			O-Phos-phate (EA data) (mg l^{-1})	Nitrate (EA data) (mg l^{-1})	BCD (EA data) (mg l^{-1})	DO (EA data) (%)	Ammonia (EA data) (mg l^{-1})	254 nm	340 nm	410 nm	Exci-tation (nm)	Emis-sion (nm)	Inten-sity	Exci-tation (nm)	Emis-sion (nm)	Inten-sity			
41	DERWENT AT LINTZFORD BRIDGE	mean	0·361	2·842	1·817	96	0·098	0·162	0·062	0·022	235	426	204	335	431	105	47	43	97
		stdev	0·163	0·509	0·930	9	0·047	0·050	0·016	0·009	0	10	26	4	7	22	5	13	22
42	PONT BURN AT 85318	mean	1·309	6·496	1·723	95	0·277	0·112	0·029	0·015	241	422	206	334	421	125	68	46	129
		stdev	0·639	2·323	0·578	7	0·822	0·036	0·007	0·007	7	8	46	7	3	36	19	10	39
43	DERWENT AT SHOTLEY BRIDGE	mean	0·031	1·131	1·575	100	0·035	0·226	0·069	0·028	236	436	206	334	437	107	33	31	72
		stdev	0·009	0·579	0·526	5	0·013	0·039	0·010	0·007	0	10	25	9	7	18	2	8	15
44	DERWENT AT ALLENSFORD	mean	0·025	0·829	1·450	102	0·030	0·222	0·073	0·031	235	437	205	334	437	103	26	30	61
		stdev	0·006	0·458	0·528	7	0·001	0·036	0·009	0·006	0	6	19	6	8	17	3	7	11
45	WHARNLEY BURN AT RIVER DERWENT	mean	0·033	2·205	1·533	96	0·035	0·086	0·029	0·013	235	424	156	336	432	73	27	29	52
		stdev	0·016	0·863	0·440	6	0·017	0·050	0·012	0·008	1	8	26	3	7	23	3	9	6
46	WALLISH WALLS BURN AT ALLENSFORD	mean	0·170	8·986	1·600	100	0·061	0·245	0·063	0·024	239	420	362	331	423	223	68	25	111
		stdev	0·117	3·232	0·628	8	0·048	0·056	0·022	0·012	5	5	30	11	8	31	12	13	19
47	HORSLEYHOPE BURN AT ROAD BRIDGE	mean	0·024	0·433	1·550	99	0·033	0·171	0·057	0·024	235	431	187	336	436	92	26	41	71
		stdev	0·005	0·152	0·493	6	0·036	0·088	0·031	0·013	0	5	37	6	10	28	6	10	20
48	DERWENT AT EDDYS BRIDGE	mean	0·023	0·761	1·625	99	0·031	0·274	0·095	0·039	233	435	203	333	438	107	26	33	56
		stdev	0·006	0·247	0·466	8	0·002	0·031	0·010	0·005	4	7	22	9	8	9	4	12	10
49	DERWENT AT CARRICKS PICNIC SITE	mean	0·024	0·427	1·625	102	0·031	0·185	0·066	0·029	235	432	165	339	442	86	20	31	57
		stdev	0·005	0·288	0·483	9	0·002	0·110	0·043	0·018	3	5	22	2	8	19	5	10	26
51	BOLTS BURN AT CONFLUENCE	mean	0·021	0·475	1·575	97	0·030	0·100	0·029	0·014	232	428	126	335	436	57	20	41	55
		stdev	0·006	0·307	0·648	6	0·000	0·055	0·021	0·012	6	9	22	8	8	18	5	17	11
52	EAST ALLEN AT HUNTWELL	mean	0·032	0·212	1·496	101	0·030	0·217	0·071	0·028	234	436	207	337	446	104	24	40	66
		stdev	0·013	0·037	0·318	4	0·000	0·130	0·048	0·021	2	3	35	4	8	24	5	15	15

53	WEST ALLEN AT	mean	0·024	0·196	1·493	98	0·030	0·224	0·068	0·026	234	433	156	337	443	82	21	38	42
	SCRAITHOLE	stdev	0·006	0·001	0·561	6	0·000	0·118	0·037	0·018	2	5	24	7	5	21	3	5	17
54	SOUTH TYNE AT	mean	0·026	0·206	1·471	101	0·031	0·257	0·090	0·035	235	438	168	341	446	103	21	34	54
	GARRIGILL FORD	stdev	0·011	0·032	0·439	4	0·003	0·147	0·054	0·022	0	6	34	2	5	32	4	12	13
55	BLACK BURN AT	mean		0·191	1·750	99	0·031	0·288	0·105	0·041	235	438	181	340	442	90	16	26	40
	INTACK	stdev		0·006	1·270	3	0·001	0·145	0·059	0·025	1	8	30	0	3	27	3	13	11
56	SOUTH TYNE AT	mean	0·027	0·220	1·550	102	0·031	0·255	0·081	0·036	236	435	172	338	446	95	19	27	46
	ALSTON	stdev	0·017	0·043	0·332	5	0·002	0·131	0·050	0·022	5	8	29	4	6	29	3	13	9
57	NENT AT ALSTON	mean	0·021	0·296	1·486	103	0·034	0·128	0·037	0·017	234	432	178	334	437	80	23	45	66
		stdev	0·003	0·128	0·357	4	0·016	0·083	0·030	0·016	3	4	41	7	10	27	5	19	20
58	SOUTH TYNE AT	mean	0·029	0·229	1·671	103	0·032	0·254	0·091	0·037	234	436	177	335	441	93	20	33	52
	EALS	stdev	0·012	0·048	0·468	5	0·006	0·142	0·054	0·023	2	8	37	8	8	29	3	16	13
60	PARK BURN AT	mean	0·036	0·368	1·950	100	0·033	0·475	0·169	0·062	238	436	271	339	443	179	30	29	67
	PARK VILLAGE	stdev	0·017	0·198	0·768	5	0·007	0·223	0·061	0·031	7	9	27	2	8	37	8	15	21
61	TIPALT BURN AT	mean	0·042	0·825	1·900	83	0·046	0·631	0·231	0·083	239	439	227	339	443	163	26	25	52
	A69 ROAD	stdev	0·023	0·254	0·497	4	0·014	0·437	0·166	0·059	7	15	51	2	9	47	10	16	27
	BRIDGE																		
62	SOUTH TYNE AT	mean	0·034	0·310	1·715	104	0·031	0·429	0·169	0·062	238	436	210	341	445	143	24	26	60
	HAL TWHISTLE	stdev	0·033	0·141	0·478	9	0·005	0·218	0·095	0·033	7	15	33	1	7	53	9	13	29
63	ALLEN AT	mean	0·038	0·337	1·767	104	0·032	0·348	0·119	0·046	235	435	203	338	438	117	27	38	86
	ALLENBANKS	stdev	0·029	0·184	0·385	9	0·006	0·276	0·107	0·042	1	6	41	4	8	35	8	15	17
64	TYNE AT CREW	mean	0·042	0·803	1·977	102	0·036	0·318	0·107	0·039	235	434	213	335	443	118	28	37	65
	HALL	stdev	0·028	0·844	0·626	9	0·010	0·127	0·058	0·020	1	8	44	6	9	33	5	10	17

Table II. Correlation coefficients between fluorescence, absorbance and chemical water quality parameters. A-254, A-340 and A-410 are absorbances at 254, 340 and 410 nm, respectively. H-ex, H-em and H-I are the excitation and emission wavelengths and 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; T-280, T-220 and Tyrosine are intensities of the tryptophan-like and tyrosine-like fluorescence centres

			Phosphate	Nitrate	BOD	DO	Ammonia	A-254 nm	A-340 nm	A-410 nm	H-ex
Spearman's rho	Phosphate	Correlation coefficient	1·000	0·652**	0·550**	−0·415**	0·792**	−0·274*	−0·295*	−0·315*	0·415**
		Sig. (2-tailed)		0·000	0·000	0·001	0·000	0·033	0·021	0·013	0·001
		N	61	61	61	61	61	61	61	61	61
	Nitrate	Correlation coefficient	0·652**	1·000	0·259*	−0·423**	0·706**	−0·588**	−0·612**	−0·629**	0·027
		Sig. (2-tailed)	0·000		0·042	0·001	0·000	0·000	0·000	0·000	0·834
		N	61	62	62	62	62	62	62	62	62
	BOD	Correlation coefficient	0·550**	0·259*	1·000	−0·335**	0·492**	−0·062	−0·083	−0·085	0·392**
		Sig. (2-tailed)	0·000	0·042		0·008	0·000	0·653	0·522	0·510	0·002
		N	61	62	62	62	62	62	62	62	62
	DO	Correlation coefficient	−0·415**	−0·423**	−0·335**	1·000	−0·580**	0·140	0·146	0·164	−0·335**
		Sig. (2-tailed)	0·001	0·001	0·008		0·000	0·279	0·257	0·202	0·006
		N	61	62	62	62	62	62	62	62	62
	Ammonia	Correlation coefficient	0·792**	0·706**	0·492**	−0·560**	1·000	−0·271*	−0·313*	−0·327**	0·347**
		Sig. (2-tailed)	0·000	0·000	0·000	0·000		0·033	0·013	0·009	0·006
		N	61	62	62	62	62	62	62	62	62
	A-254 nm	Correlation coefficient	−0·274*	−0·588**	−0·062	0·140	−0·271*	1·000	0·968**	0·984**	0·371**
		Sig. (2-tailed)	0·033	0·000	0·633	0·279	0·033		0·000	0·000	0·003
		N	61	62	62	62	62	63	63	63	63
	A-340 nm	Correlation coefficient	−0·295*	−0·612**	−0·083	0·146	−0·313*	0·988**	1·000	0·994**	0·359**
		Sig. (2-tailed)	0·021	0·000	0·522	0·257	0·013	0·000		0·000	0·004
		N	61	62	62	62	62	63	63	63	63
	A-410 nm	Correlation coefficient	−0·315*	−0·629**	−0·085	0·184	−0·327**	0·964**	0·994**	1·000	0·355**
		Sig. (2-tailed)	0·013	0·000	0·510	0·202	0·000	0·000	0·000		0·004
		N	61	62	62	62	62	63	63	63	63
	H-ex	Correlation coefficient	0·415**	0·027	0·392**	−0·335**	0·347**	0·371**	0·359**	0·355**	1·000
		Sig. (2-tailed)	0·001	0·834	0·002	0·008	0·006	0·003	0·004	0·004	
		N	61	62	62	62	62	63	63	63	63
	H-em	Correlation coefficient	−0·431**	−0·733**	−0·196	0·227	−0·468**	0·810**	0·834**	0·844**	0·257*
		Sig. (2-tailed)	0·001	0·000	0·123	0·077	0·000	0·000	0·000	0·000	0·042
		N	61	62	62	62	62	63	63	63	63

H-1	Correlation coefficient	0·619**	0·633**	0·407**	−0·330**	0·583**	−0·250*	−0·296*	−0·322*	0·257*
	Sig. (2-tailed)	0·000	0·000	0·001	0·009	0·000	0·048	0·019	0·010	0·042
	N	61	62	62	62	62	63	63	63	63
F-ex	Correlation coefficient	−0·282*	−0·705**	−0·114	0·106	−0·368**	0·736**	0·770**	0·773**	0·338**
	Sig. (2-tailed)	0·028	0·000	0·379	0·414	0·003	0·000	0·000	0·000	0·007
	N	61	62	62	62	62	63	63	63	63
F-em	Correlation coefficient	−0·498**	−0·788**	−0·205	0·278*	−0·518**	0·840**	0·860**	0·869**	0·157
	Sig. (2-tailed)	0·000	0·000	0·110	0·029	0·000	0·000	0·000	0·000	0·219
	N	61	62	62	62	62	63	63	63	63
F-I	Correlation coefficient	0·434**	0·166	0·435**	−0·371**	0·432**	0·483**	0·434**	0·404**	0·693**
	Sig. (2-tailed)	0·000	0·196	0·000	0·003	0·000	0·000	0·000	0·001	0·000
	N	61	62	62	62	62	63	63	63	63
T-280	Correlation coefficient	0·670**	0·854**	0·348**	−0·333**	0·662**	0·863**	−0·684**	−0·699**	0·043
	Sig. (2-tailed)	0·000	0·000	0·006	0·008	0·000	0·000	0·000	0·000	0·739
	N	61	62	62	62	62	63	63	63	63
Tyrosine	Correlation coefficient	0·335**	0·504**	0·252*	−0·109	0·334**	−0·820**	−0·810**	−0·806**	−0·264*
	Sig. (2-tailed)	0·008	0·000	0·048	0·400	0·008	0·000	0·000	0·000	0·037
	N	61	62	62	62	62	63	63	63	63
T-220	Correlation coefficient	0·533**	0·741**	0·300*	−0·196	0·528**	−0·736**	−0·753**	−0·760**	−0·058
	Sig. (2-tailed)	0·000	0·000	0·018	0·128	0·000	0·000	0·000	0·000	0·653
	N	61	62	62	62	62	63	63	63	63

Table II. (*Continued*)

			H-em	H-I	F-ex	F-em	F-I	T-280	Tyrosine	T-220
Spearman's rho	Phosphate	Correlation coefficient	−0·431**	0·619**	0·282*	−0·496**	0·434**	0·670**	0·335**	0·533*
		Sig. (2-tailed)	0·001	0·000	0·028	0·000	0·000	0·000	0·008	0·000
		N	61	61	61	61	61	61	61	61
	Nitrate	Correlation coefficient	−0·733**	0·633**	−0·705**	−0·788**	0·156	0·854**	0·604**	0·741**
		Sig. (2-tailed)	0·000	0·000	0·000	0·000	0·196	0·000	0·000	0·000
		N	62	62	62	62	62	62	62	62
	BOD	Correlation coefficient	0·198	0·407**	−0·114	−0·205	0·435**	0·348**	0·252*	0·300*
		Sig. (2-tailed)	0·123	0·001	0·379	0·110	0·000	0·006	0·048	0·018
		N	62	62	62	62	62	62	62	62
	DO	Correlation coefficient	0·227	−0·330**	0·106	0·278*	−0·371**	−0·333**	−0·109	−0·196
		Sig. (2-tailed)	0·077	0·009	0·414	0·029	0·003	0·008	0·400	0·126
		N	62	62	62	62	62	62	62	62
	Ammonia	Correlation coefficient	−0·468**	0·583**	−0·368**	−0·518**	0·432**	0·652**	0·334**	0·528**
		Sig. (2-tailed)	0·000	0·000	0·003	0·000	0·000	0·000	0·006	0·000
		N	62	62	62	62	62	62	62	62
	A-254 nm	Correlation coefficient	0·810**	−0·250*	0·738**	0·840**	0·483**	−0·653**	−0·620**	−0·736**
		Sig. (2-tailed)	0·000	0·048	0·000	0·000	0·000	0·000	0·000	0·000
		N	63	63	63	63	63	63	63	63
	A-340 nm	Correlation coefficient	0·834**	−0·296*	0·770**	0·860**	0·434**	−0·684**	−0·810**	−0·753**
		Sig. (2-tailed)	0·000	0·019	0·000	0·000	0·000	0·000	0·000	0·000
		N	63	63	63	63	63	63	63	63
	A-410 nm	Correlation coefficient	0·844**	−0·322*	0·773**	0·869**	0·404**	−0·699**	−0·508**	−0·760**
		Sig. (2-tailed)	0·000	0·010	0·000	0·000	0·001	0·000	0·000	0·000
		N	63	63	63	63	63	63	63	63
	H-ex	Correlation coefficient	0·257*	0·257*	0·338**	0·157	0·693**	0·043	−0·264*	−0·058
		Sig. (2-tailed)	0·042	0·042	0·007	0·219	0·000	0·739	0·037	0·653
		N	63	63	63	63	63	63	63	63
	H-em	Correlation coefficient	1·000	−0·536**	0·792**	0·877**	0·104	−0·808**	−0·778**	−0·843**
		Sig. (2-tailed)		0·000	0·000	0·000	0·417	0·000	0·000	0·000
		N	63	63	63	63	63	63	63	63

H-1	Correlation coefficient	−0·536**	1·000	−0·428**	−0·576**	0·586**	0·833**	0·434**	0·757**
	Sig. (2-tailed)	0·000		0·000	0·000	0·000	0·000	0·000	0·000
	N	63	63	63	63	63	63	63	63
F-ex	Correlation coefficient	0·792**	−0·428**	1·000	0·833**	0·200	−0·705**	−0·685**	−0·725**
	Sig. (2-tailed)	0·000	0·000		0·000	0·116	0·000	0·000	0·000
	N	63	63	63	63	63	63	63	63
F-em	Correlation coefficient	0·877**	−0·576**	0·833**	1·000	0·108	−0·855**	−0·767**	−0·867**
	Sig. (2-tailed)	0·000	0·000	0·000		0·400	0·000	0·000	0·000
	N	63	63	63	63	63	63	63	63
F-I	Correlation coefficient	0·104	0·586**	0·200	0·106	1·000	0·207	−0·278*	0·054
	Sig. (2-tailed)	0·417	0·000	0·116	0·400		0·104	0·027	0·674
	N	63	63	63	63	63	63	63	63
T-280	Correlation coefficient	−0·808**	0·833**	−0·705**	−0·855**	0·207	1·000	0·726**	0·945**
	Sig. (2-tailed)	0·000	0·000	0·000	0·000	0·104		0·000	0·000
	N	63	63	63	63	63	63	63	63
Tyrosine	Correlation coefficient	−0·778**	0·434**	−0·685**	−0·767**	−0·278*	0·726**	1·000	0·841**
	Sig. (2-tailed)	0·000	0·000	0·000	0·000	0·027	0·000		0·000
	N	63	63	63	63	63	63	63	63
T-220	Correlation coefficient	−0·843**	0·757**	−0·725**	−0·867**	0·054	0·945**	0·841**	1·000
	Sig. (2-tailed)	0·000	0·000	0·000	0·000	0·674	0·000	0·000	
	N	63	63	63	63	63	63	63	63

* Correlation significant at the 0·05 level (2-tailed).
** Correlation significant at the 0·01 level (2-tailed).

wavelength. Absorbance correlates positively with humic and fulvic-like emission wavelengths (Table II) and negatively with humic and fulvic-like intensities, the latter due to inner-filtering as described earlier. Within the fluorescence dataset, the protein-like fluorescence intensities (the tyrosine and two tryptophan-like centres) have a strong correlation between each other, and weak correlations with humic and fulvic-like intensities.

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). 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, as described earlier, three sites within the dataset have statistically outlying data, from non-fluorescent propylene glycol deicer (sites 1 and 2) and a combination of mine water, treated sewage effluent 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. This is shown in Figure 2 and Table III. Tryptophan-like fluorescence intensity at the 280 nm excitation/350 nm emission fluorescence centre correlates with both phosphate ($r = 0{\cdot}80$) and nitrate ($r = 0{\cdot}87$), whereas tryptophan-like fluorescence intensity at the 220 nm excitation/350 nm emission wavelength centre correlates with BOD ($r = 0{\cdot}85$), ammonia ($r = 0{\cdot}70$) and dissolved oxygen ($r = -0{\cdot}65$). Figure 2 shows that in all cases there are a large number (about 50 of the 62 sample sites) of essentially good water quality sample sites that cluster with low values of both tryptophan-like fluorescence intensity and the respective chemical water quality parameter. Sites of poorer water quality (about 12 sites) have higher tryptophan-like fluorescence intensity and concentration of the measured chemical water quality parameter, which either form a second cluster of data points (for example dissolved oxygen and ammonia) or a linear trend (for example nitrate). These sites are those on the urban rivers. The Ouseburn (sites 1–3) has known sewerage water quality issues (Baker *et al*., 2003), the River Don (site 34 and 35) also has sewerage water quality issues, the River Team (sites 36–39) is impacted by wastewater treatment works effluent, sewerage overflows, mine water and landfill leachate, and the lower reaches of the Derwent (sites 40–41) are downstream of wastewater treatment works effluents. In addition, the Pont Burn (site 42), although not urban in land-use, is a small tributary that has a wastewater treatment works that provides a significant proportion of total flow, and one small agricultural watercourse (Wallish Walls Burn, site 46) also occasionally features in the poor water quality cluster.

That some correlations between chemical water quality parameters are stronger with the tryptophan-like fluorescence centre at excitation wavelength of 220 nm, and others with the centre at 280 nm, is significant. The centre at 280 nm excitation will be within the tail of fluorescence from the fulvic-like peak when this centre has high fluorescence intensity, and would be expected to have a stronger correlation with pollutant sources that have significant fluorescence intensities in both the tryptophan-like and fulvic-like fluorescence centres. Baker (2001, 2002b) demonstrates that this can be the case for sewage effluents rather than for farm wastes, as the latter have predominantly protein-like fluorescence centres. Therefore the correlation between this tryptophan-like fluorescence centre and nitrate and phosphate confirms that all three are tracing sewage-derived DOM. Sites with highest nitrate and phosphate concentrations are typically those on rivers downstream of wastewater treatment works and where these effluents provide a significant proportion of total flow (sites 36–38 and 40–42). Urban rivers with sewerage issues affecting water quality (sites 1–3, 34–35), as opposed to those sites with a significant volume of treated effluents, also have high nitrate, phosphate and tryptophan-like fluorescence, but cluster with slightly higher tryptophan-like fluorescence intensity and lower nitrate and phosphate concentration. Wallish Walls Burn (site 46), the only site with agricultural-derived water quality issues, only exhibits elevated nitrate concentration as might be expected.

The correlation between BOD and the tryptophan-like fluorescence intensity at the 220 nm excitation wavelength centre suggests that BOD has a stronger correlation with tryptophan-like fluorescence alone. Assuming that the fulvic-like peak is predominantly derived from the relatively stable DOM pool, our

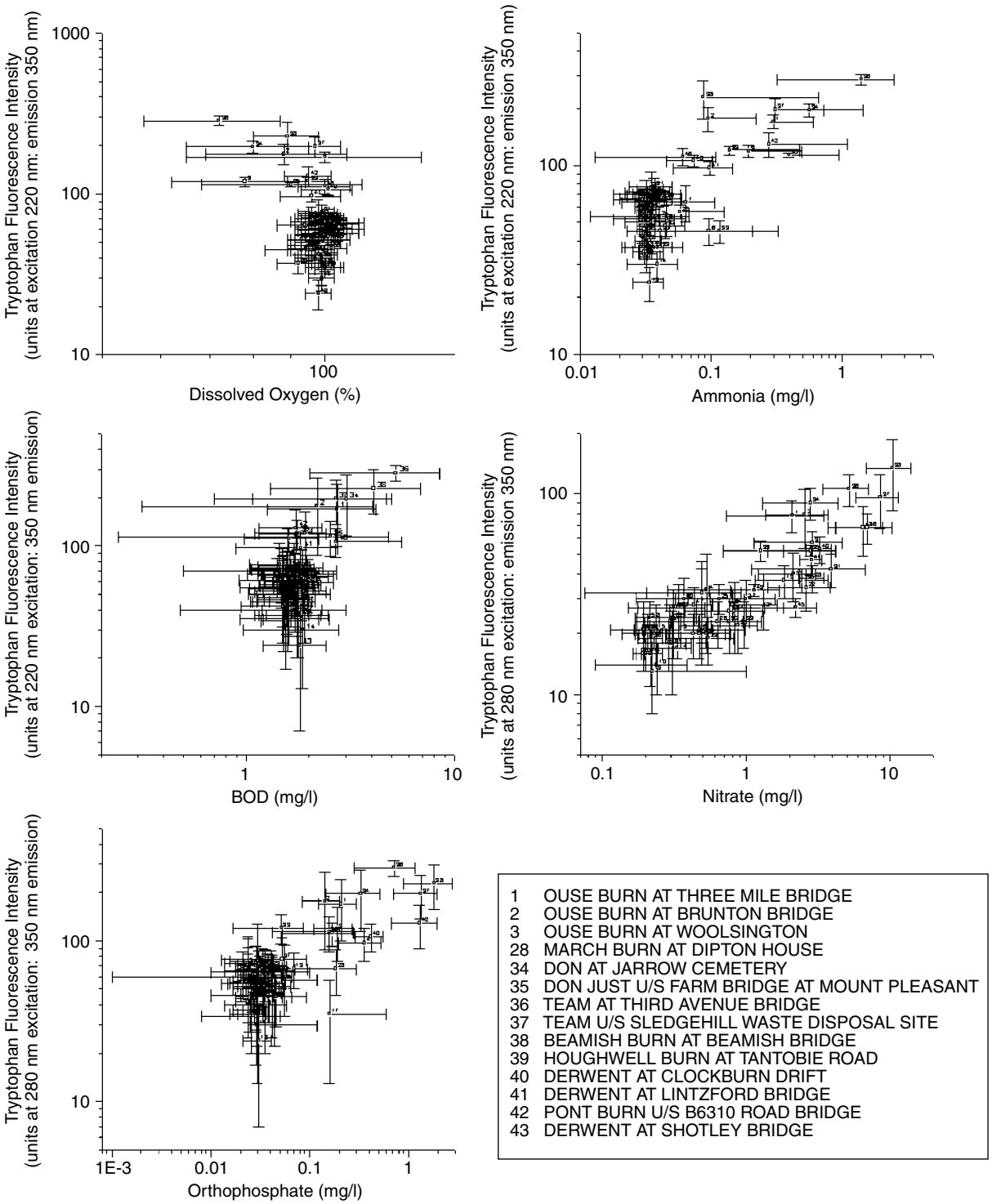

Figure 2. Graphs of tryptophan-like fluorescence intensity against chemical water quality determinant. Results are presented in log–log format due to the skewed data distribution. Error bars are the 1σ standard deviation, and sample site numbers are those from Table I

Table III. Regression equations between fluorescence and chemical water quality parameters. Abbreviations for humic-like, fulvic-like, tryptophan-like and tyrosine-like fluorescence are as Table II. Correlation coefficients are shown for both simple linear regression with one correlant, and for the stepwise regression equation shown

Chemical water quality determinand	Stepwise regression	Stepwise r	Single parameter r
Phosphate	$-8{\cdot}55 + 0{\cdot}01808T_{280} + 0{\cdot}02536F_{ex} - 0{\cdot}002082H_I$	0·894	0·799
Nitrate	$-0{\cdot}006798 + 0{\cdot}112T_{280} - 0{\cdot}07127\text{Tyro}$	0·935	0·874
BOD (all data)	$-1{\cdot}586 + 0{\cdot}0569\text{Tyro} + 0{\cdot}01269F_{em}$	0·754	0·675
BOD (excluding sites 1, 2)	$-13{\cdot}467 + 0{\cdot}01578T_{220} + 0{\cdot}03232F_{em}$	0·903	0·846
DO (EA data)	$102{\cdot}578 - 0{\cdot}07328T_{220}$	0·646	0·646
Ammonia (all data)	$-0{\cdot}224 + 0{\cdot}002733H_I - 0{\cdot}002171F_I$	0·845	0·784
Ammonia (excluding site 36)	$-0{\cdot}04166 + 0{\cdot}0016T_{220}$	0·703	0·703

correlation therefore suggests that the tryptophan-like fluorescence centre is related to the bioavailable or labile DOM pool. Six sites on the urban rivers Ouseburn, Team and Don form a high BOD cluster in Figure 2, but in general the range of BOD in the Tyne catchment is not great enough to assess the strength of the BOD–tryptophan-like fluorescence intensity relationship. The majority of sample sites have a BOD that is close to the detection limits of the technique and BOD error bars reflect analytical errors as much as natural sample variability.

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 shown in Table III, 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.

Finally, we investigated the relationship between the mean and standard deviation of tryptophan-like fluorescence intensity and the GQA for each site for the year 2002 as performed by the Environment Agency. The GQA is scored from A to F, where A is the highest water quality and F the lowest, and is based on the dissolved oxygen, BOD and ammonia results. The results for a site are averaged for a 36-month period centred on the year of interest, and percentiles are calculated. These are compared with limits set for each of the six grades. A grade is assigned to the length of river (which the sampling site represents) according to the lowest grade achieved by any of the three determinants. Results are presented in Figure 3, 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. Sites where the fit between GQA and tryptophan-like fluorescence intensity is weakest include the Wallish Walls Burn (site 46: graded E, mean tryptophan-like fluorescence intensity of

111 units), which is known to be affected by intermittent agricultural pollution and which was probably undersampled during our fluorescence sampling programme of six samples per year. Two other sites of interest are the Tarset and Chirdon Burns (sites 13 and 14: graded B, mean tryptophan-like fluorescence intensity of 24 and 30 units), where the fluorescence results suggest a grade of A would be more appropriate. The two sites have the most coloured water and drain peaty uplands and their GQA score of B is based on a failure to meet BOD requirements. The discrepancy is therefore likely to be due to incorrect GQA grading of the rivers due to the difficulty in measuring BOD at low concentrations. However, despite these discrepancies, 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

Within our study of the Tyne catchment, mean annual tryptophan-like fluorescence intensity at the 280 nm excitation/350 nm emission centre has a strong correlation with mean annual nitrate and phosphate concentrations, and mean annual BOD has a strong correlation with mean annual tryptophan-like fluorescence intensity at the 220 nm excitation/350 nm emission centre. But this is only true when three outlying sites are removed. Non-fluorescent DOM such as the >60 mg l^{-1} deicer pollution event in January 2003 on two sites on the Ouseburn obscures any correlation—use of tryptophan-like fluorescence intensity as a chemical water quality determinant would not be possible in catchments where similar pollution issues occur, although we feel these would not be common. The effect of a combination of treated wastewater and landfill leachate at one site on the River Team also impacts on the ammonia–tryptophan-like fluorescence relationship due to the unusually high ammonia concentrations and fluorescence intensities at the site. This is probably due to the landfill leachate, as high ammonia values are also observed on the upstream tributary impacted by the leaking landfill. Caution would therefore be needed in catchments where landfill leachate was a significant pollutant. Weaker but statistically significant correlations are observed between fluorescence and ammonia and dissolved oxygen, but again tryptophan-like fluorescence intensity is still the most significant correlant.

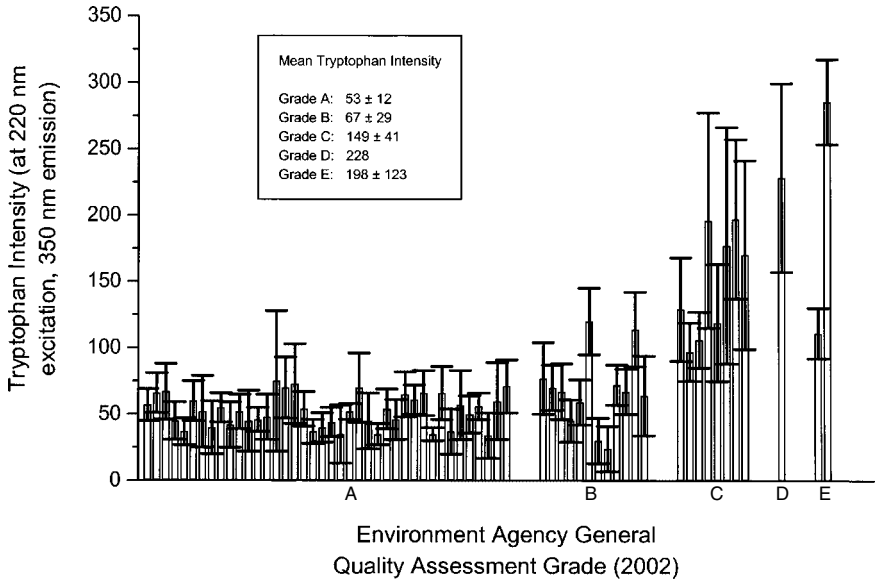

Figure 3. Comparison of tryptophan-like fluorescence intensity and Environment Agency General Quality assessment for 2002

In the Tyne catchment, with the exception of the airport deicer 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 in the screened dataset suggest 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 catchments (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 min) also permits the real-time analysis of waters. Technological advances within spectrophotometry will continue to increase the portability of equipment, already field-based fluorescence analysis is possible (Hart and Jiji, 2002) and the potential therefore exists for tryptophan-like fluorescence to also be used by both environmental regulators and operators of consented discharges.

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