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Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy

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Abstract

Dissolved organic matter fluorescence, absorbance and dissolved organic carbon were measured from source to sea in the River Tyne catchment, of ~2935 km^2 and encompassing areas of contrasting land use. The catchment has three major tributaries: the North Tyne which has good water quality, high dissolved organic carbon concentrations and visible water colour from the high proportion of peat in its upper catchment; the South Tyne which has good water quality with typical riverine dissolved organic carbon concentrations and drains from limestone uplands; and the Derwent, a more urbanized catchment which is increasingly impacted by treated sewage effluent discharges towards its mouth. Thirty sample sites, 23 along the three main tributaries and seven within the estuary, were sampled on six occasions over the period 2002–2003. High absorbance at 340 nm and dissolved organic carbon concentration identify N Tyne waters due to the peaty headwaters, but no downstream trends in these parameters are observed in any of the tributaries, in contrast to the estuary where a rapid decrease is observed in both. Fluorescence in contrast demonstrated downstream trends in both intensity and wavelength, especially in the Derwent as it is increasingly impacted by anthropogenic dissolved organic matter. Elevated protein-like fluorescence intensity also fingerprints sewage effluent within the estuary. The absorbance coefficient at 340 nm was found to have the strongest correlation to dissolved organic carbon concentration, greater than all fluorescence intensity parameters measured. However, fluorescence analysis permits the source of the dissolved organic matter to be determined, and therefore has implications for understanding its fate in estuaries and the ocean.

Keywords: Dissolved organic matter; Absorbance; Fluorescence; Dissolved organic carbon; Tyne

1. Introduction

Dissolved organic matter (DOM) is ubiquitous in aquatic systems, and it has a wide range of molar masses and chemical structures that give it a multi-functional role in the natural environment (Frimmel, 1998a) Characterization of DOM and its reactivity from source to sea is important to understand its role in ecosystem functioning (Benner, 2002; Sinsabaugh and Findlay, 2003), mobilisation of organic and inorganic pollutants (Xiaoying, 2001), photodegradation (Frimmel, 1998b), its affect on drinking water treatment (Watts et al., 2001) and carbon budgeting

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(Freeman et al., 2001). Approximately 25% of DOM is well characterized; this comprises amino acids, nucleic acids, carbohydrates, hydrocarbons, fatty acids and phenolic compounds (Thomas, 1997) thus the vast majority of DOM remains uncharacterized. Estimates of the amount of humic substances in aquatic DOM are in the region of 50–70% (Thurman, 1985).

What happens to terrestrial DOM in the ocean is a question to which the answer is fundamental to understanding the global carbon cycle and its anthropogenic perturbations (Hedges et al., 1997). Riverine dissolved organic carbon (DOC) shows a generally conservative behaviour during estuarine mixing, although in some cases flocculation may account for a small degree of removal (Sholkovitz, 1976; Mantoura and Woodward, 1983; Amon and Benner, 1996). In the absence of significant removal processes, terrigenous DOC would, therefore be expected to accumulate in the oceans. However, although the global annual discharge of terrigenous DOC to the ocean is ~0.25 Pg, evidence for a major terrigenous DOM component in the ocean is lacking (Hedges et al., 1997; Benner, 2002). Rivers alone discharge sufficient DOC to support the turnover of DOC throughout the ocean (Williams and Druffel, 1987) and riverine DOM is generally thought to be resistant to biodegradation and has a chemical composition indicating it to be primarily soil derived and highly degraded (Hedges et al., 1994). DOM in the oceans, however, has a stable carbon isotopic composition indicating a predominantly marine origin (Druffel et al., 1992) and this is supported by low concentrations of lignin—derived phenols indicative of a minor terrestrially derived component (Opsahl and Benner, 1997).

The question of the fate of terrigenous DOM in the ocean thus is to be resolved and has been outlined as one of the major topics of ongoing and future research about the cycling of DOM in marine systems (Benner, 2002). This seems particularly pertinent in light of a recent study by Freeman et al. (2001) who observed a 65% increase in the DOC concentration in freshwater draining from upland catchments in the UK over the past 12 years. The increase has been suggested to result from either or both of rising temperatures and catchment land use change driving the process by stimulating the export of DOC from peatlands (Worrell et al., 2003). With global climate change the rate of movement is likely to increase further if global temperatures increase; thus resulting in a key terrestrial carbon store relocating to the oceans. Traditional methods of characterizing DOM (e.g. isotopic or molecular level characterization) often need large sample volumes and long and complex handling procedures, which increases the possibility for contamination or chemical alteration of the original material (Coble, 1996). This severely reduces the number of samples that can be collected, and impedes the regular analysis of short-term variability (Coble, 1996), and is also comparatively expensive. Therefore as an alternative, optical properties have been used to distinguish DOM of different origins and to quantify DOM fluxes to the oceans.

DOM has distinctive spectrophotometric properties in terms of both absorption of light and fluorescence. As well as strong absorption in ultra-violet light, much dissolved organic matter fluoresces (FDOM). FDOM has been investigated for many years to characterise marine and estuarine DOM (for example, see Mopper and Schultz, 1993; Coble, 1996; Mayer et al., 1999). Recent research has increasingly investigated riverine FDOM. FDOM is a potential tracer of DOM fractions in aquatic systems. McKnight et al. (2001, 2003) used FDOM to trace microbial and terrestrial derived fulvic material in alpine/sub-alpine catchment in USA; Thoss et al. (2000) used FDOM to trace organic matter fractions in six catchments of contrasting land use in North Wales, and Newson et al. (2001) used FDOM properties of colored river water as a natural tracer in a small peaty sub catchment of the River Eden, N England.

Importantly, recent advances in fluorescence spectrophotometry permit the rapid (approx. 1 min) detection of FDOM at a wide range of both excitation and emission wavelengths to produce an excitation–emission matrix or EEM (Coble, 1996). 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 centers that are attributed to both natural DOM groups such as humic and fulvic-like material, as well as fluorescent proteins (for a review of possible fluorescence centers see Coble, 1996 and for typical EEMs see Baker, 2001). Protein fluorescence centers observed in EEMs are at the

same wavelengths as those of tryptophan (fluorophore T) and tyrosine (fluorophore B), although it is not known how these fluorescence centers relate to the structure of DOM. Fluorescence centers ascribed to humic-like (fluorophore A) and fulvic-like (fluorophore C) material occur at higher emission wavelengths. Again, the exact relationship between fluorescence properties and biogeochemical structure of the organic matter is unknown, but it is recognised that the fluorescence is generated by highly substituted aromatic nuclei, extensive conjugation and high molecular weight compounds (Senesi et al., 1989) and potential fluorophores such as benzene, phenol and toluene containing compounds have all been identified in DOM isolates. Fluorophores A and C have elongated contours, as opposed to rounded contours as would be expected from a pure organic fluorophore, and this is a function of the presence of multiple fluorophores or inter-molecular energy transfer (Coble et al., 1990). Fluorophore A has undergone less investigation than fluorophore C, although it is known that FDOM can contain either one or both of the fluorophores that generate fluorophores A and C (Boehme and Coble, 2000). The rapid analysis time of FDOM EEMs (approx. 1 min compared to approx. 20 min in the 1990s and approx. 1 day in the 1980s) now permits the analysis of a much larger number of aquatic samples than previously possible.

Here we present the results of an FDOM sampling program from source to sea in the Tyne river-estuary system, N England, which was studied to exploit the differences in DOM compositions from contrasting catchment properties. The Tyne catchment contains three tributary rivers. The North Tyne has very high dissolved organic matter concentrations as it receives humic-rich waters draining areas of thick (up to 10 m) blanket peat, whereas the South Tyne drains relatively pristine moorland in limestone headwaters with thinner soils, and is therefore less organic rich than the North Tyne. The North and South Tyne converge downstream to form the Tyne River, which supplies >90% of the total river discharge into the Tyne estuary. The third tributary is that of the Derwent: this has similar headwaters to the South Tyne but is increasingly urbanized downstream and is impacted by a number of wastewater treatment plant discharges which impact water quality. The Derwent discharges into the mid-estuary. Therefore, the Tyne can be regarded as a system with simple end member mixing, ideally suited for a study of estuarine transport to the sea, as well as one of contrasting catchment and DOM properties that are relevant to a wide range of regional and global river-estuary systems.

2. Materials and methods

2.1. Study site

DOM optical characteristics were investigated from source to sea in a large river catchment in northern England. The River Tyne has a catchment area of 2935 km^2 and comprises two main tributaries, the North and South Tyne that meet near Hexham (Fig. 1). The North Tyne rises in the Cheviot Hills near the Scottish Border, the South Tyne in the Cumbrian Pennines. The two rivers join to form the River Tyne, which flows in an easterly direction to Wylam, where it becomes tidal for the last ~33 km of its course. Another major tributary of the Tyne is the River Derwent, which enters the Tyne in its tidal section. The Tyne catchment generally consists of rolling hills, rising to 500 m in the north and 800 m in the south, with the Tyne valley reaching a downstream width of several kilometres. Rainfall in the catchment varies from <700 mm in the eastern lowlands to 1500 mm in the uplands, with precipitation falling as snow in the uplands in winter. Soils in the catchment are dominated by large areas of peat in the uplands, which provides a substantial store of organic carbon and a source of colored water, and with stagno-gleys in the majority of the remaining areas. In general the soils are slow draining, and are underlain by shallow or low permeability aquifers, leading to the hydrology of many sub-catchments being dominated by surface runoff with rapid response to rainfall as a result of saturation excess. However, both the North Tyne and Derwent contain reservoirs and downstream flow is highly regulated. The largest reservoir is that at Kielder, which impounds water from 240 km^2 of the North Tyne. Fig. 2 presents discharge data over the study period from the Tyne at Bywell and Derwent at Rowlands Gill. Discharge on the River Tyne at this location is a combination of the regulated North and unregulated South Tyne rivers,

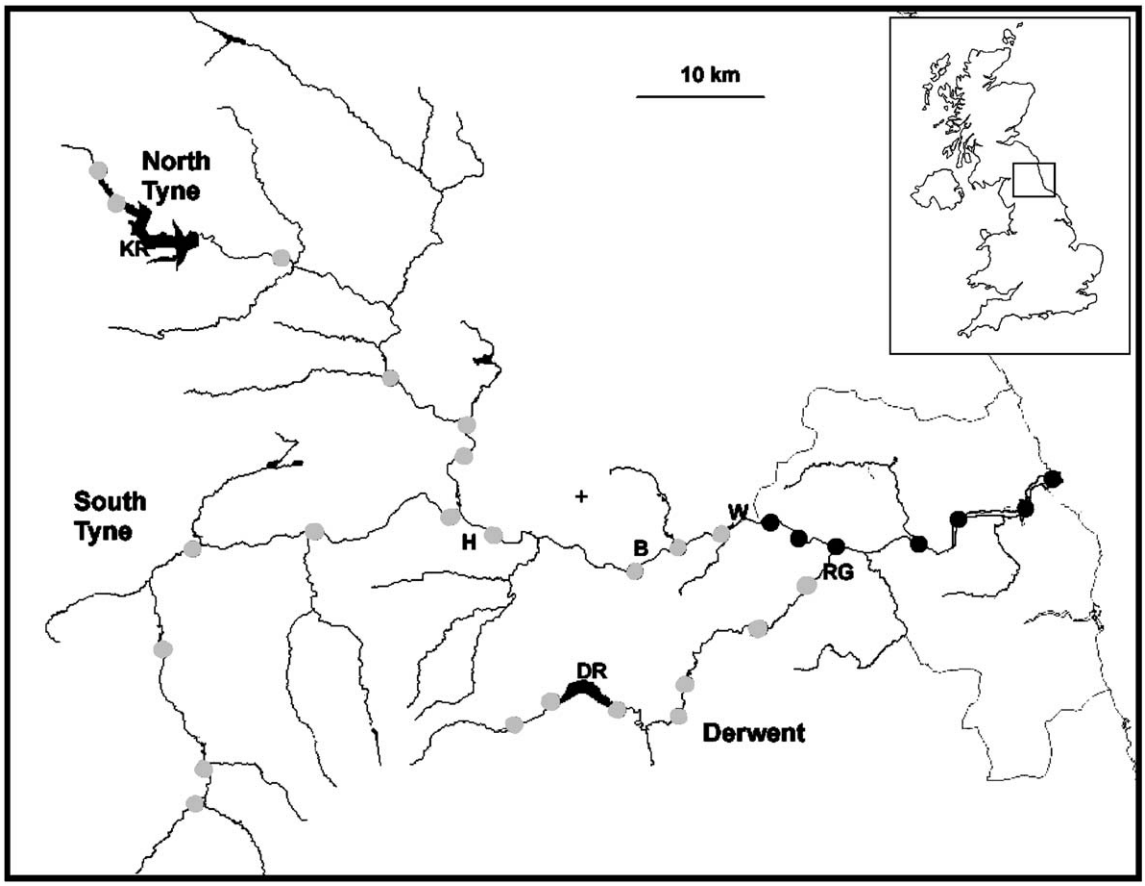

Fig. 1. Map of the Tyne Catchment. Sample locations are shown by circles. Rivers N and S Tyne and Derwent are labelled: the confluence of the N and S Tyne occurs just west of Hexham (H). Discharge data is from Bywell (B) and Rowlands Gill (RG). The tidal limit is at Wylam (W). Kielder Reservoir (KR) and Derwent Reservoir (DR) are also shown, as is the county line for Tyne and Wear (light grey) and urban areas (grey shading). The cross-hair marks 55° N 2° W, north is up.

and demonstrates storm flow dominating in winter and baseflow with Kielder compensation releases in summer and the occasional summer storm event. The Derwent hydrograph similarly has minimum compensation flow release superimposed by winter rainfall events.

Upstream of Bywell, the catchment is predominantly rural, whereas below Bywell an urban population of approximately 750 000 influences the water quality of the river, with 214 consented discharges from sewage treatment works, 126 consented industrial discharges and 492 storm sewage discharges. River lengths of poor water quality are predominantly in lowland urbanized parts of the catchment (including the lower reaches of the river Derwent) with many sewage inputs and without substantial upland clean water supplies to dilute them. The River Tyne drains into the Tyne Estuary, a partially mixed mesotidal estuary. Industrial fluxes to the lower estuary are declining, however, it continues to receive significant urban waste; primarily treated sewage from Howdon sewage works (approx. 9.1×10^7 m^3 $year^{-1}$).

2.2. Sample collection

River and estuarine water samples were collected during two separate but parallel field programs operating from the spring of 2002 through to spring 2003; the river program was part of a wider study to

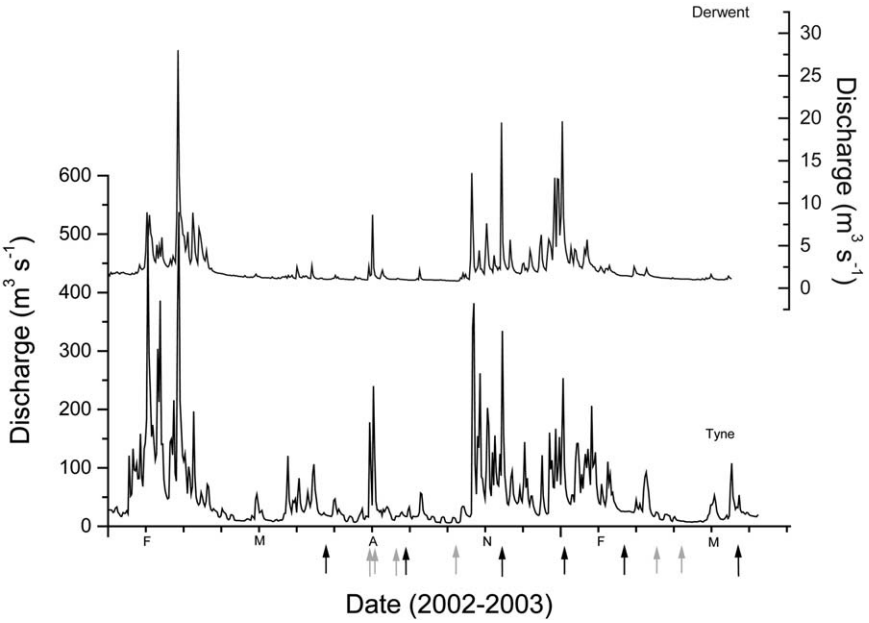

Fig. 2. Discharge data for River Tyne (Bywell) and River Derwent (Rowlands Gill). Timing of the water sampling trips also shown by arrows: black for freshwater sampling and grey for estuarine sampling.

investigate the relationship between land use and water quality on the River Tyne, and the estuarine samples part of a program investigating nitrogen and carbon fluxes in the Tyne estuary. Differences in these wider objectives mean that subtle differences in sampling protocols and dates occurred in each field program.

River samples were taken from 23 sites along Tyne and Derwent rivers every 2 months between May 2002 and May 2003. The sites are main river locations, frequently downstream samples of major sub-catchments and mid-catchment samples at points of changing land use or anthropogenic impact. Fig. 1 shows the location of the sample sites. Spectrophotometric (both absorbance and fluorescence) measurements of river water at the sample sites were taken under a range of flow regimes (58 ± 89 m^3 s^{-1}) from summer base flow (August 2002) through to winter storm flow (November 2002) and during winter low flow during extensive snow cover (January 2003). The timing of samples and the relationship to river flow is shown in Fig. 2. Water samples were collected in 30 ml aged polypropylene bottles, which had been pre-cleaned in 10% HCl and distilled water. Samples were refrigerated upon return from the field, filtered (Whatman GF/C precombusted (450 °C) glass microfibre filter papers) and analyzed within 24 h.

Estuarine water samples were collected on six transects between July 2002 and April 2003 conducted from RV *Bernicia* and a rigid inflatable boat. Samples were taken from seven locations within the estuary on each transect, and these are used here. Estuarine samples were taken on different tidal states, although predominantly over the high tide to permit boat access to the inner estuary and to minimize the effect of any tidal resuspension sources of DOM in the estuary, and sampling dates are different from those of the river sampling runs. Although a wide range of river flow is covered (35 ± 34 m^3 s^{-1}) that is similar to the river sampling regime, the estuarine sampling program differed in sampling a summer rather than autumn high discharge event. Fig. 2 compares the timing of the two sampling programs and river flow data. Water samples were collected in precombusted (550 °C) glass bottles and filtered immediately through 0.7 μm (Whatman GF/F) precombusted (450 °C) filters. The filtrate was then

collected directly in a 10% HCl pre-cleaned, precombusted (550 °C) glass vial with a Teflon-lined cap and stored at 4 °C in the dark until return to the laboratory.

2.3. Determination of fluorescence

To minimise temperature effects all samples were allowed to reach laboratory temperature prior to measurement. Fluorescence measurements were undertaken using a Perkin–Elmer LS-50B luminescence spectrometer as described elsewhere (Baker, 2001). Validation was performed daily using a sealed water cell containing pure water and the Raman fluorophore of water at 348 nm was used to monitor instrument stability and to permit inter-laboratory comparison. Results were adjusted to a Raman fluorophore intensity of 20.0 units. Fluorescence EEMs were produced and from these seven fluorescence parameters were extracted. The parameters associated with humic-like FDOM were fluorophores A and C intensity and emission wavelengths. Excitation wavelengths were not extracted as excitation was scanned at 5 nm steps as the best compromise between sample resolution and analysis time, and so this parameter showed little variation. The parameters associated with protein-like fluorescence were the intensities of fluorophores T and B. Fluorophore T intensity, that associated with tryptophan-like fluorescence, was measured at both 220 and 280 nm excitation centers. Fluorophore B intensity, which is associated with tyrosine-like fluorescence, was measured only at the 220 nm excitation center. Emission wavelengths of the three fluorescence centers were recorded but found to be invariant, and so not considered further.

2.4. Determination of absorbance

For riverine and estuarine samples, the absorption coefficient at 340 nm (a_{340}) was determined using Lightwave (WPA) and Uvikon 923 (Kontron Instruments) spectrophotometers, both to provide data from this established technique and to provide a check for inner-filtering effects (when highly absorbent DOM quenches fluorescence, resulting in a decrease in intensity; Mobed et al., 1996; Ohno, 2002). Distilled deionised water was used as a reference, and absorbance readings were corrected when necessary for long-term baseline drift. Samples from the peat dominated North Tyne catchment were visibly colored and often exhibited inner-filtering, with a_{340} maxima of >70 m^{-1} which decreased fluorophore C intensities by >10%. No inner-filtering correction was applied as we wished to investigate the use of the raw FDOM signature.

2.5. Determination of total organic carbon

DOC analysis used the HTCO method incorporating a Shimadzu TOC Analyzer and a platinized alumina catalyst. Samples were acidified to pH ~ 2 with HCl and either run directly following collection or were frozen until analysis. The acidified samples (pH ~ 2) were sparged for 8 min at 75 or 100 ml/min^{-1} with either ultra-pure air or ultra-pure oxygen to remove inorganic carbon from samples prior to the measurement. The mean of three to five injections of 100 μl is reported for every sample and precision, described as a coefficient of variance (C.V.), was <2% for the replicate injections.

3. Results

Riverine and estuarine FDOM results are not based on paired samples, as waters were sampled on different days over the period 2002–2003. Such sampling methods permit a statistical analysis of FDOM variations from source to sea, based on the mean and standard deviation of each parameter measured at each sample site over the study period.

3.1. Comparison of river sub-catchment and estuarine FDOM, a_{340} and DOC

Table 1 presents the data for all 30 sample sites. DOC is lowest on the South Tyne and Derwent, with values typically lower than 700 μM, whereas higher values were found in the North Tyne with DOC ~ 1000 μM. All fall within the range of typical global (Volk et al., 1997; Lobbes et al., 2000) and regional (Worrell et al., 2003) riverine values: the high DOC on the North Tyne is due to the extensive and locally thick (>10 m) peat cover that dominates this sub-catchment. Estuarine DOC is a similar concentration to the non-tidal Tyne in the inner estuary, and

Table 1
Summary DOC, absorbance and fluorescence data for the Tyne catchment and estuary

Distance from source (km)	Sample Site	DOC (μM)	Absorption coefficient a_{340} (m^{-1})	Fluorescence						
				Peak A		Peak C		Peak T_{280} Intensity (units)	Peak B Intensity (units)	Peak T_{220} Intensity (units)
				Emission λ (nm)	Intensity (units)	Emission λ (nm)	Intensity (units)			
South Tyne										
19	Intack	325±242	24±14	438±8	161±30	442±3	90±27	16±3	26±13	40±11
22	Alston	275±208	21±12	435±8	172±29	446±6	95±29	19±3	27±13	46±9
33	Eals	275±225	21±12	436±6	177±37	441±8	93±29	20±3	33±16	52±13
44	Haltwhistle	617±458	39±22	438±15	210±33	445±7	143±53	24±9	26±13	60±29
55	Crew Hall	592±592	25±13	434±8	213±44	443±9	118±33	28±5	37±10	65±17
69	Warden Bridge	650±417	37±21	436±9	211±41	445±8	133±42	30±5	35±15	57±12
North Tyne										
14	U/S Kielder	683±475	51±44	443±9	142±40	449±11	103±36	14±4	24±14	34±17
16	Reservoir	1275±108	63±5	440±11	167±23	448±7	136±15	16±3	14±8	35±5
35	Tarset	1225±142	65±14	438±8	170±24	449±4	141±27	17±2	17±11	35±8
52	Wark	1167±350	65±27	440±6	179±51	449±8	148±35	18±5	15±5	37±10
60	Barrasford	1142±400	57±17	435±7	192±31	449±8	151±37	20±3	21±15	37±9
64	Chollerford	1100±400	67±34	442±9	183±45	449±6	150±40	19±5	16±8	37±17
Tyne										
72	Hexham	942±467	53±25	442±10	198±33	443±3	143±34	23±6	22±10	46±15
87	Bywell	958±475	53±25	439±10	197±32	446±7	148±40	22±6	23±11	45±14
91	Ovingham	1008±525	54±24	435±14	198±31	443±6	149±43	23±7	20±11	48±17
95	Wylam	1033±508	46±31	443±10	225±41	445±7	167±33	23±6	21±12	45±16
Derwent										
7	U/S Bolts Burn	383±233	18±11	437±8	158±29	444±6	84±24	19±4	31±15	44±11
10	U/S reservoir	342±183	15±10	432±5	165±22	442±8	86±19	20±5	31±10	57±26
16	D/S reservoir	483±58	22±2	433±7	203±22	438±8	107±9	26±4	33±12	56±10
24	Allensford	408±83	17±2	437±6	205±19	437±8	103±17	26±3	30±7	61±11
27	Shotley Bridge	408±83	16±2	436±10	206±25	437±7	107±16	33±2	31±8	72±15
37	Lintzford Bridge	292±208	12±4	426±10	204±26	431±7	105±22	47±8	43±13	97±22
44	Clockburn Drift	275±158	10±4	427±9	212±28	428±7	105±21	53±8	49±13	106±21
Estuary										
101–102	Ryton	1427±834	60±37	430±9	352±108	442±4	288±150	30±6	24±8	83±38
105–106	Lemington Gut	1106±623	43±36	434±8	320±105	439±7	230±139	38±12	34±18	97±32
107–108	Scotswood	1150±641	42±33	432±7	318±105	440±5	224±123	35±9	30±30	104±40
114	St. Peters marina	824±477	27±25	420±4	260±124	440±7	160±112	37±6	39±8	100±23
118	Wallsend	789±452	21±24	425±10	210±144	438±7	123±113	36±6	44±12	107±18
122	Howdon	667±267	16±15	416±12	224±90	426±13	113±113	62±21	100±50	212±97
127	Tynemouth	427±348	13±16	413±2	159±129	431±10	83±90	33±10	45±9	121±25

For sample locations see Fig. 1: the confluence of the N and S Tyne occurs 70 km from the source and the tidal section starts at 98 km. The Derwent is tidal at 51 km from the source and enters the Tyne estuary after 53 km, where the Tyne is 109 km from the source. Freshwater samples were collected in June, August and November 2002, and January, March and May 2003, and estuarine samples were collected in July, August and October 2002 and March and April 2003. The absorption coefficient (a_{340})=2.303 A_{340} d^{-1}, where A_{340} is the absorbance at 340 nm and d is the optical path length. Fluorophore T is recorded as the intensity maximum at ~220 nm excitation/~350 nm emission (T_{220}) and ~280 nm excitation/~350 nm emission (T_{280}). Fluorophore B is the intensity maximum at ~220 nm excitation/~305 nm emission (a second centre at 280 nm excitation is obscured by the Raman line of water and not recorded). Fluorophore A is the intensity maximum between 220 and 250 nm excitation and 400 and 480 nm emission. Fluorophore C is the intensity maximum between 300 and 380 nm excitation and 400 and 480 nm emission.

declines to ~400 μM at the outer estuary, a finding higher than estuaries elsewhere (Miller, 1999; Rochelle-Newall and Fisher, 2002) due to the higher DOC concentrations from the Tyne riverine input, and the input of Howdon sewage works in the outer estuary. The standard deviation of sample DOC concentrations near the estuarine mouth is large due to low DOC values at low river flow (approx. 150 μM) and high DOC concentrations at high river flow where a large source of riverine derived DOC can be observed at the outer estuary (approx. 850 μM). a_{340} follows a similar trend to DOC: Fig. 3 shows DOC against a_{340} for all sample sites and demonstrates a strong correlation (r=0.89, n=30); a_{340} was found to be the best proxy for DOC from all the optical measurements taken.

FDOM results also demonstrate variability between sub-catchments and the estuary. Fluorophore C emission wavelength is highest for the North and South Tyne catchments, with mean emission wavelengths between 441 and 449 nm. On the Derwent, mean fluorophore C emission wavelength varies between 428 and 444 nm, and in the estuary the mean fluorophore C emission wavelengths range from 426 to 442 nm. Fluorophore C emission wavelengths have been demonstrated elsewhere to correlate with the molecular weight or aromaticity of DOM (Coble, 1996) suggesting that the Tyne has higher molecular weight/more aromatic DOM that the Derwent, and that this is in turn of higher molecular weight/aromaticity than DOM from Howdon sewage treatment works or that of marine origin near the estuarine mouth. Fluorophore C emission wavelengths exhibit a similar trend to fluorophore A emission wavelength. All fall within the range of previously published results: for example, at the Coalburn Experimental Catchment, fluorophore C emission wavelength ranged from 434 to 448 nm at a site which has thick peat cover and is 5 km west of the N Tyne catchment (Newson et al., 2001), whereas Baker (2002a) observed fluorophore C emission wavelengths of between 410 and 423 nm in a small highly urbanized catchment of the Ouseburn.

The intensity of FDOM fluorophores A and C exhibit little variability between the river sub-catchments. Mean fluorophore A intensity ranges from 142 to 225 units for all river sample sites, and mean fluorophore C intensity ranges from 84 to 167 units for all sites. Within the estuary, fluorophore A and C intensities have both a greater range and are higher

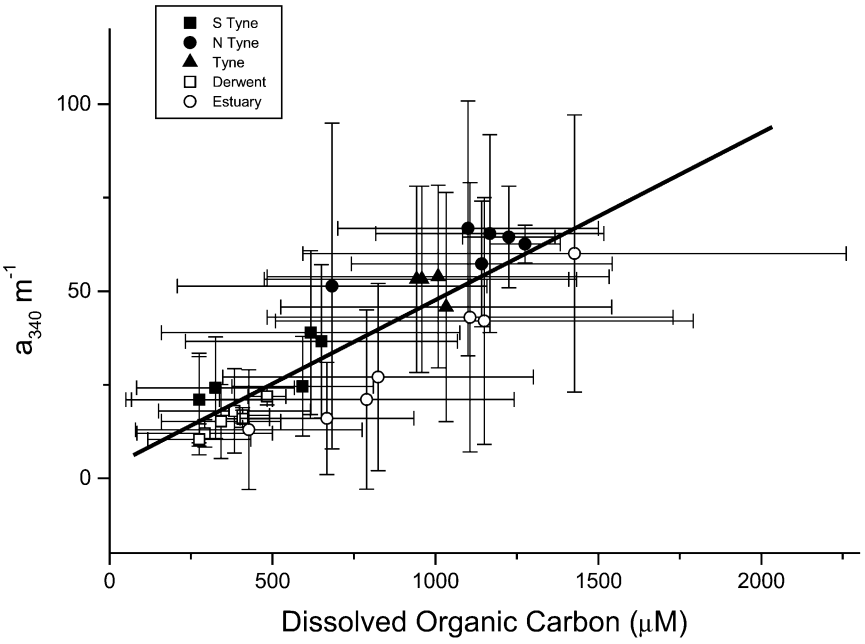

Fig. 3. Relationship between DOC and a_{340}.

(159 units–352 units and 83 units–288 units, respectively). The higher fluorescence intensities occur in the inner estuary and correlate with high a_{340} and DOC and are a result of sampling within the summer high discharge event (Fig. 2) that was a DOC flushing event. No similar event was sampled within the riverine sample runs. The two fluorophores correlate strongly (r=0.90, n=30), suggesting that they have a similar FDOM source, and reasonably strongly with a_{340} (r=0.71 and 0.93 for fluorophores A and C, respectively, n=30) and DOC (r=0.44 and 0.74 for fluorophores A and C, respectively, n=30). The range of fluorophore A and C intensities observed in the river samples is within that observed elsewhere (Baker, 2002a).

The protein-like fluorescence fluorophores T_{220}, T_{280} and B strongly correlate with each other (r>0.84, n=30) and weakly correlate with the fluorophores A and C (0.51>r>−0.11; n=30), DOC (r<−0.15, n=30) and a_{340} (r<0.02, n=30), suggesting a different FDOM source for these centers and one that has greater fluorescence efficiency. Fluorophore T_{220} has similar values of ~15–25 units in the river sample sites, with the exception of downstream samples on the Derwent, where fluorescence intensity increases to ~30–50 units. This pattern is mirrored in both T_{280} and fluorophore B fluorescence intensities. Protein-like fluorescence has been demonstrated to increase with increased anthropogenic FDOM from sewage (both treated and untreated) and farm wastes (Baker, 2001, 2002b) and the lower Derwent is affected by multiple treated sewage inputs. Estuarine fluorophore T_{220}, T_{280} and B fluorescence intensities are higher than the river samples, with the exception of the lower Derwent sites, and particularly high values are observed at the Howdon site, which is within the effluent plume from the Howdon sewage treatment works. High protein-like fluorescence intensities observed in the estuary are probably due to increased biological activity in the estuary as elevated protein-like fluorescence has been shown in regions with relatively high levels of biological activity (e.g. photic zones) (Mopper and Schultz, 1993).

3.2. Source to sea FDOM on the River Tyne

Fig. 4 shows the mean and standard deviation values of all FDOM parameters measured, together with DOC and a_{34D}. Several overall trends are apparent in the data. Variability of all parameters decreases on the North Tyne downstream of the Kielder Reservoir due to the regulated flow. In contrast, variability at the estuarine sites is in general greater than that at the river sites, especially for the parameters that correlate with DOM concentration (DOC, a_{340}, fluorophore A and C intensity). In part this is due to sampling on different tidal states, such that an individual sample site has a wide range of salinities: this is evidenced by a decrease in variability in DOC as the outer estuary is approached, where salinities are less variable and generally more representative of North Sea water. The high variability is also due to the sampling of the high DOM concentration summer discharge event in July 2002: this explains the high variability in DOM concentration parameters.

The lack of significant a_{340} and DOC trends from source to estuary is typical of riverine systems, although it must also be considered that it could be a function of the use of different sampling regimes at the riverine and estuarine sites. Within the river subcatchments no downstream trends are visible within the range of seasonal variability, although the N and S Tyne are differentiated as detailed earlier by both parameters. Within the estuary, both a_{340} and DOC decrease rapidly, with the decrease in DOC in ~33 km of estuary as great as the total range of DOC in the ~160 km of N and S Tyne rivers; thus indicative of the mixing of the DOM rich riverine water with the comparatively DOM poor North Sea water. In contrast, FDOM fluorophores A and C do not differentiate the two sub-catchments, although intensities also rapidly decline in the estuary. The lower fluorescence intensities in the N Tyne are a function of inner-filtering effects, as the DOC concentrations on the river are above the ~800 μM threshold for DOM where inner-filtering may be considered to become an issue (Kalbitz and Geyer, 2001). Serial dilutions on N Tyne samples confirmed a fluorescence quenching of 10–30%: without this inner-filter effect higher fluorophore A and C intensities would be observed on the N Tyne.

FDOM fluorophores A and C emission wavelengths also show no downstream trends within the non-tidal Tyne, and no differences between the N and S Tyne sub-catchments, but a rapid decrease in wavelength in the estuary. The stability of the wave-

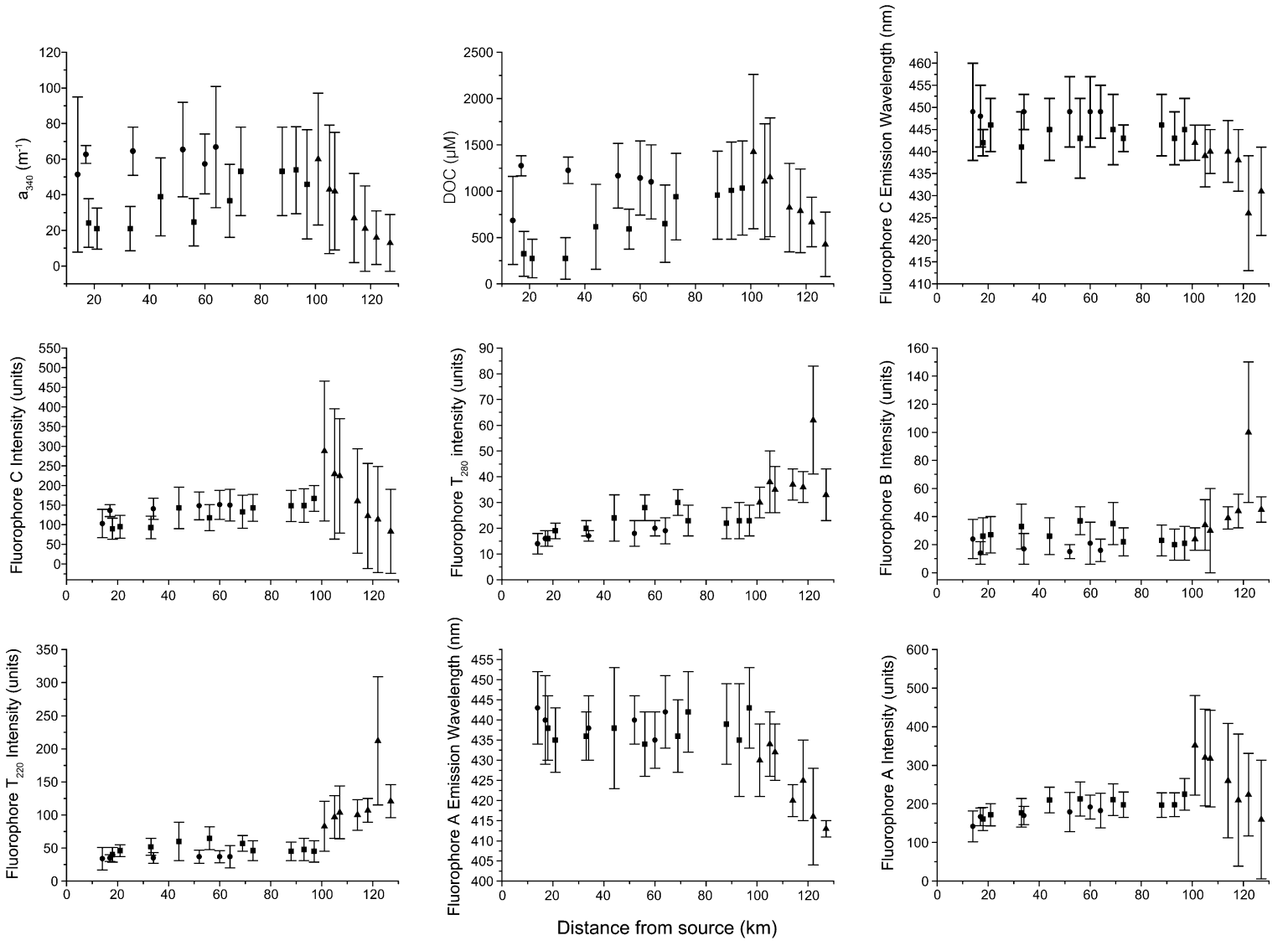

Fig. 4. Source to sea fluorescence, a_{340} and DOC for the River Tyne. Distances are from the source of the N Tyne (circles): the S Tyne (squares) source is at ~4 km. Estuarine samples are shown by triangles.

lengths along the length of the Tyne is not unexpected given the dominance of peat derived DOC that would be expected to be highly degraded and relatively stable. However, this contrasts with the decrease in emission wavelength of fluorophore C in the estuary. The observed shift in fluorophore C emission wavelength to a shorter emission maximum in marine samples has been described in previous studies (Coble, 1996; Del Castillo et al., 1999). This observed blue shift in the emission maxima of fluorophore C towards higher salinity, is indicative of a loss of longer wavelength lower energy transitions consistent with a decrease in aromaticity (Coble, 1996) and has been defined as a distinct fluorophore in marine waters (fluorophore M). Such a decrease in aromaticity is consistent with photochemical and microbial breakdown of terrigenous DOM, its mixing with or substitution with a less aromatic marine DOM, or a combination of these processes. Peak A also shows a decrease in emission wavelength maxima towards the marine end of the estuary, this is potentially again due to photochemical or microbial breakdown of terrigenous DOM, its mixing or substitution with a less aromatic marine form of DOM, or some combination of these processes. However, it may also be a manifestation of the anthropogenic source of DOM from Howdon sewage treatment works, which appears to be less aromatic than terrigenous DOM.

Protein-like FDOM fluorophores T_{220}, T_{280} and B also exhibit no downstream trends within the N and S Tyne. FDOM intensities are low for all three centers: in contrast a rapid increase in fluorescence is observed in the estuary. Because estuaries are typically zones of enhanced biological production it is not unsurprising that elevated protein fluorescence is shown within these samples. It is presumed this dissolved protein-like fluorescence results from exudation and spillage of cell contents during cell growth and grazing (Mayer et al., 1999). Wikner et al. (1999) showed an increased degradation of riverine DOC at elevated salinities and outlined that an enhancement of primary production is due to the inputs of riverine inorganic nutrients, and algal products then stimulate the bacterioplankton. Furthermore Stepanauskas et al. (1999) outlined how an enhanced utilization of organically bound riverine nutrients may stimulate bacterial production and then through mineralization by the microbial loop, the growth of algae. The mechanism behind increased utilization of DOM at increased salinities remains unclear, however, a number of potential methods can be suggested. Mulholland (1981) showed that significant changes in high molecular weight DOM occurred with increasing ionic strength, such as flocculation. The coiling of dissolved humic substances (de Haan et al., 1987) and changes in sorption mechanisms have also been documented at elevated ionic strength (Taylor, 1995). Flocculation has been described to increase the utilization of DOC (Tranvik and Sieburth, 1989) and the coiling of macromolecules at elevated ionic strength might enhance their transfer through the bacterial cell wall into the periplasmic space (Stepanauskas et al., 1999). Increased protein-like fluorescence in the estuarine samples, however, could also be due to protein-like marine rich fluorescence coming from North Sea waters simply mixing with the comparatively protein-like fluorescence poor riverine water. Additionally, the Howdon sewage treatment works sample site, has significantly higher protein-like fluorescence intensities than all other sites. This Howdon sewage treatment works plume water could also elevate protein-like fluorescence within the estuary, again simply through estuarine mixing processes or a combination of these outlined processes could clearly lead to the elevated protein-like fluorescence within the estuary.

3.3. Source to sea FDOM on the River Derwent

Fig. 5 presents the downstream trends in FDOM, DOC and a_{340} from source to sea. Similar to the Fig. 4, the effects of the Derwent Reservoir can be seen in a decrease in variability of all parameters downstream of the reservoir due to flow regulation. Similarly, none of the parameters had a significant influence on the estuary because of the relatively low discharge of the Derwent when compared to the Tyne (Fig. 2). No downstream trends in DOC or a_{340} were visible, this in contrast with a slight downstream increase in fluorophore A and C intensity. This suggests a downstream increase in fluorescence efficiency, which could indicate a change in DOM structure. Over the same distance, significant increases in fluorophore T_{220}, T_{280} and fluorophore B intensity are observed, with downstream values equal or greater than estuarine intensities. High protein-like fluorescence intensities have been associated with human and farm

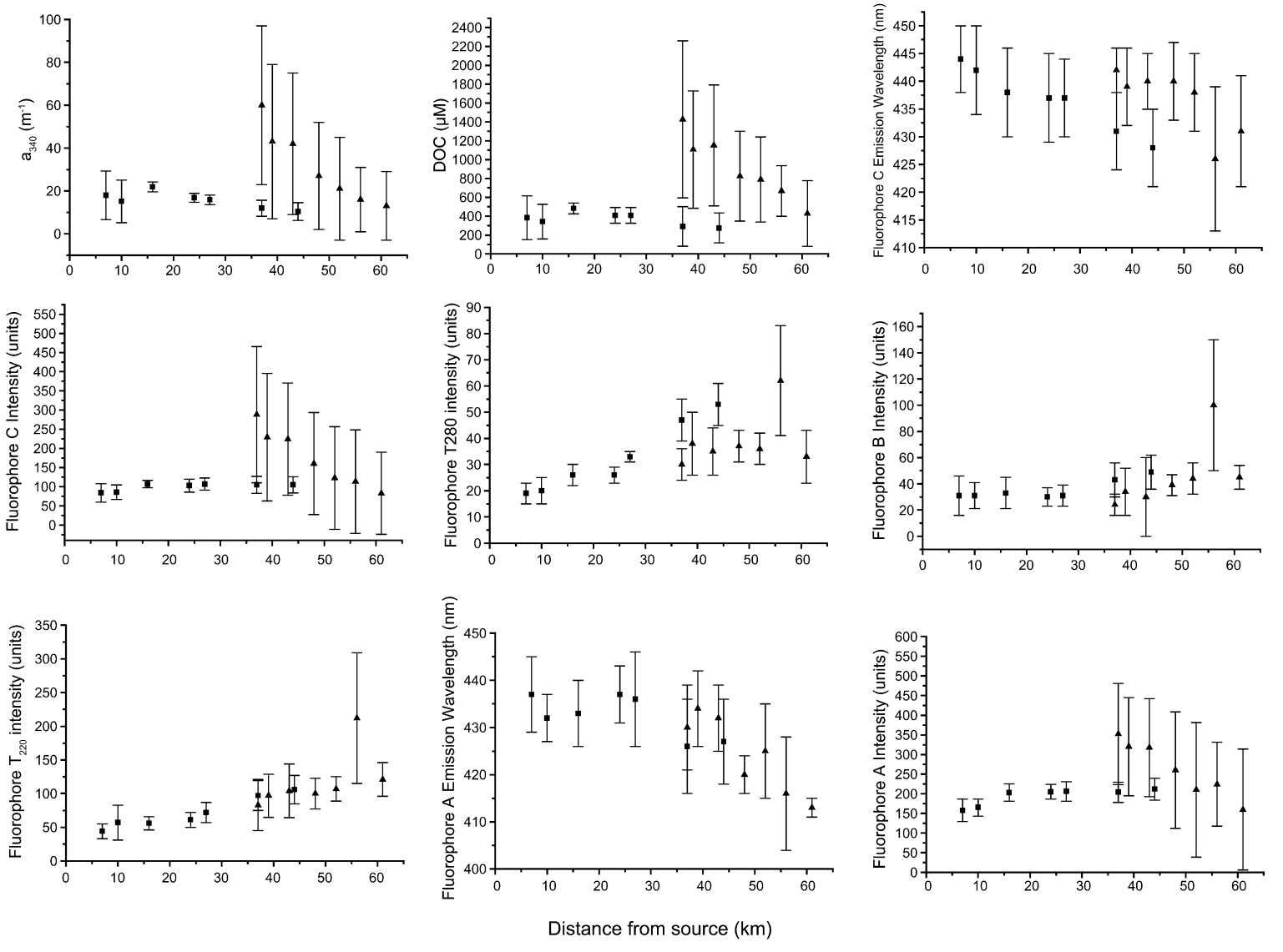

Fig. 5. Source to sea fluorescence, a_{340} and DOC for the River Derwent. Distances are from the source of the Derwent. Derwent freshwater samples are squares, estuarine samples are shown by triangles.

wastes, and the Derwent is increasingly impacted by treated wastewater discharges that would have high protein-like fluorescence intensities (Baker, 2001). This anthropogenic FDOM contribution could also explain the corresponding slight downstream increase in fluorophore C fluorescence, as well as a significant downstream decrease in fluorophore A and C emission wavelengths. Overall the Derwent is characterized by a downstream trend of decreasing emission wavelengths of fluorophores A and C, strong increases in the intensity of fluorophores T_{220}, T_{280} and B, and no change in a_{340} and DOC. Variations in these parameters over the length of the Derwent subcatchment (approx. 50 km) are, for the fluorophore A and C emission wavelength variations and protein-like fluorescence intensities, of a similar order of magnitude to variability observed over ~33 km in the estuary.

4. Discussion

4.1. Implications for studies of DOC, colored water export and water supply

The N Tyne catchment drains the central England uplands of the Pennine Hills, which in non-limestone areas is blanketed by thick peat deposits. DOC export (determined by either absorbance or color measurements) from other major rivers in the region that drain similar peat-rich uplands has been shown to have increased by 65–100% over the last 10–20 years (Freeman et al., 2001; Worrell et al., 2003). This increase has been attributed to a combination of increasing summer temperatures and summer droughts that have permitted increased bacterial breakdown of peat due to a lower water table, as well as land use change in upland catchments such as drainage of peat land to improve its quality for grazing. Understanding any potential long term trends in carbon export by rivers has global importance in terms of understanding carbon cycling and budgets. In addition, colored water poses an operational problem for water supply companies, as treatment is required within Europe to meet a standard of 0.015 absorbance units per cm at 400 nm (20 Hazen). Treatment of colored water by chlorination produces carcinogens such as trihalomethanes that pose a potential risk to human health.

In our study, DOM in the downstream freshwater Tyne is dominated by the high DOC, high fluorophore A and C intensity and emission wavelength FDOM that derives form the upland peat soil cover in the North Tyne, which provides water that is colored and of high DOM concentration. Long-term changes in DOC export from the Tyne would, therefore be expected to be driven by factors that affect the upland peat as observed elsewhere in upland Britain, and this in turn would influence DOM throughout the North Tyne and River Tyne, where it is transported from freshwater to the estuary, and subsequently relocated to the ocean. FDOM also provides useful characterization of river water that might be used for drinking water supply: on the N Tyne and Derwent major reservoirs are used for supply purposes, and there is also an intake from the main River Tyne downstream of the N and S Tyne confluence.

Paired absorbance and fluorescence analyses, which can separate the N and S Tyne sources (fluorescence) as well as precisely determine the fluxes (absorbance) of DOM, would help elucidate the relative importance of the upland peat source of DOM in the downstream Tyne river-estuary system. Detailed long term spatial and temporal monitoring within the N Tyne would also help separate the relative contributions of land use and climate change factors on DOC export to the ocean in this catchment. Future climate change scenarios suggests that our results have a global importance for other high DOC concentration rivers with peat rich headwaters both with respect to understanding carbon export from such rivers, as well as, by understanding fluorescence properties from source to sea, their changes through time, and their relation to climate and land-use changes, allowing better planning of the location and timing of abstractions for drinking water supply.

4.2. Potential for resolving the fate of terrigenous DOM in the ocean

Traditional methods for DOM characterization rely on isolation–fractionation based techniques (ion-exchange resins, reverse osmosis, rotary evaporation; Krasner et al., 1996) and tangential flow ultrafiltration (Benner et al., 1992; Guo and Santschi, 1996) and can

provide detailed biogeochemical information with minimal sample disturbance. However all these techniques produce isolates and as such it is difficult to be confident that they are the same as in the natural environment, due to both alterations in the structure of the DOM during extraction and concentration and due to their removal from the original environment in which they were situated (Shuman, 1990; Gjessing et al., 1998; Peuravuori and Pihlaja, 1998). Although DOM isolates provide a great deal of information on the chemical composition of DOM, they might not completely reflect the actual structure, behaviour, interactions and reactivity of DOM in the natural environment. In contrast the study of FDOM from source to sea allows characterization of DOM in the natural aquatic environment.

This study clearly shows that it is possible to optically characterize DOM from different catchments (e.g. N, S Tyne and Derwent rivers) and to distinguish between DOM of different sources within the estuary (for example between terrestrial, sewage and marine). Our results demonstrate that, for example, it is possible to distinguish humic rich DOM (e.g North Tyne) by the use of absorbance (providing a measurement of flux) and fluorophore C emission wavelength (an indication of source). In contrast, to determine anthropogenically impacted DOM then protein-like fluorophores provide both source and concentration information, whereas absorbance will not provide a useful measurement of flux. Therefore our results demonstrate that future studies on a wide range of river-estuarine systems will be able to use paired fluorescence and absorbance measurements to determine both source and flux of DOM from catchment sources to the sea.

To date only a few major systems have been investigated with regards to spectrophotometric parameters, namely the Orinoco, Amazon and a number of Arctic rivers. Rapid advances in spectrophotometer technology such as developments in LED and fibre optics and especially in the potential for in situ instruments, is now allowing the true potential of these techniques to be realized. Detailed surveys based on spectrophotometric techniques might allow gradual processes, such as photochemical breakdown of DOM to be observed (Hedges et al., 1997). The use of optical characteristics as a proxy for bulk DOC concentration can allow extremely detailed spatial and temporal resolution. Furthermore in situ sensors can be left in places where few research vessels can venture (e.g. Polar winters) and Guay et al. (1999) describes the use of in situ sensors on submarines in the Arctic Ocean, so clearly vast databases can be generated to look at short-term variability. In situ fluorescence measurements have also already been used to trace the distribution and concentration of river water and terrigenous DOM in the Nordic Seas (Amon et al., 2003). This study adds further support for the correlation between the absorption of light from 300 to 355 nm as a proxy for terrigenous DOM (see Hernes and Benner, 2003 and references therein) and as such has potential for remote sensing applications. Clearly these techniques have great potential to quantify the flux of terrigenous DOM to the oceans and also to increase our understanding in the mechanisms of removal of terrigenous DOM from the oceans.

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