ORGANIC MATTER FLUORESCENCE PROPERTIES OF SOME U.K. FRESH AND WASTE WATERS

By

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ABSTRACT

Organic carbon is ubiquitous throughout the aquatic environment. It is an heterogeneous mixture of compounds, some of which are fluorescent, with allochthonous and autochthonous origins. The most common aquatic fluorophores are humic materials (peaks C and A) from degraded plant matter and protein-like material (peaks T_1 and T_2) of microbial origin. Spectral fingerprints of aquatic organic matter composition may be visualised on an excitation emission matrix (EEM) on which each fluorophore is identifiable as a characteristic peak.

Protein-like fluorescence (T_1 and T_2) is linked to bacterial activity, sewage treatment process efficiency and therefore organic matter bioavailability but its source and fluorescence response is poorly understood. In comparison, peaks C and A are widely studied and have historically been considered to be old, degraded and stable.

In this thesis I investigate the character of surface water and effluent fluorescent organic matter using EEMs. I identify the likely origins and bioavailability of common fluorophores and the applicability of fluorescence as a technique for measuring the polluting potential of organic carbon in waters. I also determine changes in sample character and organic carbon concentration, through responses of the common fluorophores, under different environmental conditions and recommend best practice for sample storage.

DEDICATION

To my family.

ACKNOWLEDGEMENTS

I have many people to thank for making the completion of this work possible:

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LIST OF COMMON ABBREVIATIONS

AFU – Arbitrary Fluorescence Units

BOD_5 – Five Day Biochemical Oxygen Demand test

CDOM – Chromophoric or Coloured Dissolved Organic Matter

CFU – Colony Forming Units

COD – Chemical Oxygen Demand

CSO – Combined Sewer Overflow

DO – Dissolved Oxygen

DOC – Dissolved Organic Carbon

DOM – Dissolved Organic Matter

EEM – Excitation Emission Matrix

EEMS – Excitation Emission Matrix Spectroscopy

GWR - Geographically Weighted Regression

IHSS – International Humic Substances Society

SFS – Synchronous Fluorescence Spectroscopy

SUVA – Specific UV Absorbance

TC – Total Carbon

TIC – Total Inorganic Carbon

TOC Total Organic Carbon

$\lambda_{ex/em}$ – Excitation/ Emission Wavelength (Peak Position)

1. INTRODUCTION

1.1. Introduction

In the last 50 years fluorescence has been used extensively in the water sciences. It has been applied to investigations of the composition, concentration, distribution and dynamics of organic matter from various sources in a range of aquatic environments. In natural waters organic matter exists in dissolved, colloidal and particulate states with dissolved organic matter (DOM) being the most studied fraction, although some emphasis has been placed upon the colloidal fraction and its importance in water chemistry (Mopper et al., 1996; Mounier et al., 1999; Patel-Sorrentino et al., 2002; Boehme and Wells., 2006; Belzile and Guo, 2006; Liu et al., 2007; Seredynska-Sobecka et al., 2007; Batchelli et al., 2009). The term dissolved organic matter is often used interchangeably with dissolved organic carbon (DOC).

Dissolved organic matter in water originates from a range of sources. Some is allochthonous and is transported to the hydrological system. Major sources of allochthonous dissolved organic carbon in surface waters are the percolation of rainwater through forest leaf litters (Hongve, 1999) and the discharge of organic carbon from soils and upland/ peat catchments (Freeman et al., 2001). It has been shown that surface water DOC concentrations have been increasing in recent decades (Monteith et al., 2007). It is proposed that increases in aquatic DOC discharge from upland/ peat soils may be the result of a combination of increasing global temperatures (Freeman et al., 2001,

Clark et al., 2009); decreased water acidity (Driscoll et al., 2003; Davies et al., 2005); changes in wetting/ drying patterns of upland peats (Worrall et al., 2004, Clark et al., 2009); Increased exudation of organic carbon from roots in CO_2 enriched atmospheres – related to increasing atmospheric CO_2 concentrations (Freeman et al., 2004) among others which are reviewed by Evans et al.,(2006).

Some DOM is autochthonous and is created in-situ through microbial activity which may be an independent source of organic matter or a recycling mechanism for allochthonous DOM. Heterotrophic respiration of DOM in aquatic systems usually exceeds the autochthonous production of DOM (Cole and Caraco, 2001). Thus, an input of allochthonous DOM is required to prevent total depletion of surface water DOM. Ultimately only around half of the organic carbon which enters the surface water system is transported to the oceans. The other half is lost to the atmosphere as CO_2 or deposited in sediments (Cole et al., 2007). The proportion of allochthonous and autochthonous DOM present within a hydrological system is influenced by water type, location and environmental conditions within and without the water body. However, Zsolnay, (2003) cautions against assuming that allochthonous aquatic DOM has the same character as the organic matter at the source e.g. soil derived DOM may differ in character from the original soil organic matter.

Human activity is also a vast source of aquatic organic matter much of which is believed to be labile, which enters water through direct or diffuse discharge to surface water, indirect leaching into groundwater and aerial dispersal.

DOM was previously thought of as simply a relatively inert product of biological activity, however, advances in technology have enabled more detailed characterisation of organic material and its reactions in water (Baker, 2002a; Clark et al., 2002; Cammack et al., 2004, Steinberg et al., 2006; Cory et al., 2007; Battin et al., 2008). The presence of DOM in water impacts on the biological and physico-chemical behaviour of the water body by affecting metal speciation and altering pH (Thacker et al., 2005). CDOM (coloured or chromophoric dissolved organic matter) absorbs radiant light from the water column, decreasing that available for photosynthesis (Ferrari et al., 1996). The presence of labile organic matter is of interest with regard to river water quality (Baker and Inverarity, 2004) as oxidation of this material imposes an oxygen demand reducing oxygen levels and therefore potentially impacting aquatic life. Some DOM constituents have been found to directly and indirectly interact with aquatic (particularly freshwater) organisms (Steinberg et al., 2006).

Despite the vast body of existing work relating to aquatic DOM there is still much to be learned. Work is ongoing to further examine the complex interactions of DOM; to determine the influence of DOM source and character on lability; to investigate the character and influence of the aquatic microbial community, its substrates, products and relationship with DOM; to identify

what interactions occur within the varying components of DOM and the relative importance of these parameters; and to further understand the processes of DOM cycling within the hydrological system.

1.2. Dissolved Organic Matter Fluorescence

Organic matter fluorescence occurs when a loosely held electron in an atom or molecule is excited to a higher energy level by the absorption of energy e.g. a photon, and fluorescence occurs when energy is lost as light as the electron returns to its original energy level (ground state). Figure 1.1 shows a representation of the energy transfer involved in the process of fluorescence. Some energy is “lost” from the excited electron by collision, non-radiative decay and other processes, prior to emission, so the energy of the emitted photon is lower than the excitation energy (the Stokes’ Shift). The wavelength at which absorption (excitation) and emission occur is specific to the molecule (Lakowicz, 1999). Aromatic organic compounds provide particularly good subjects for study by fluorescence due to the energy sharing, unpaired electron structure of the carbon ring. In the study of fluorescent organic matter those compounds that absorb light are called chromophores; those that absorb and re-emit light energy are called fluorophores (Mopper et al., 1996).

Jablonski Energy Diagram

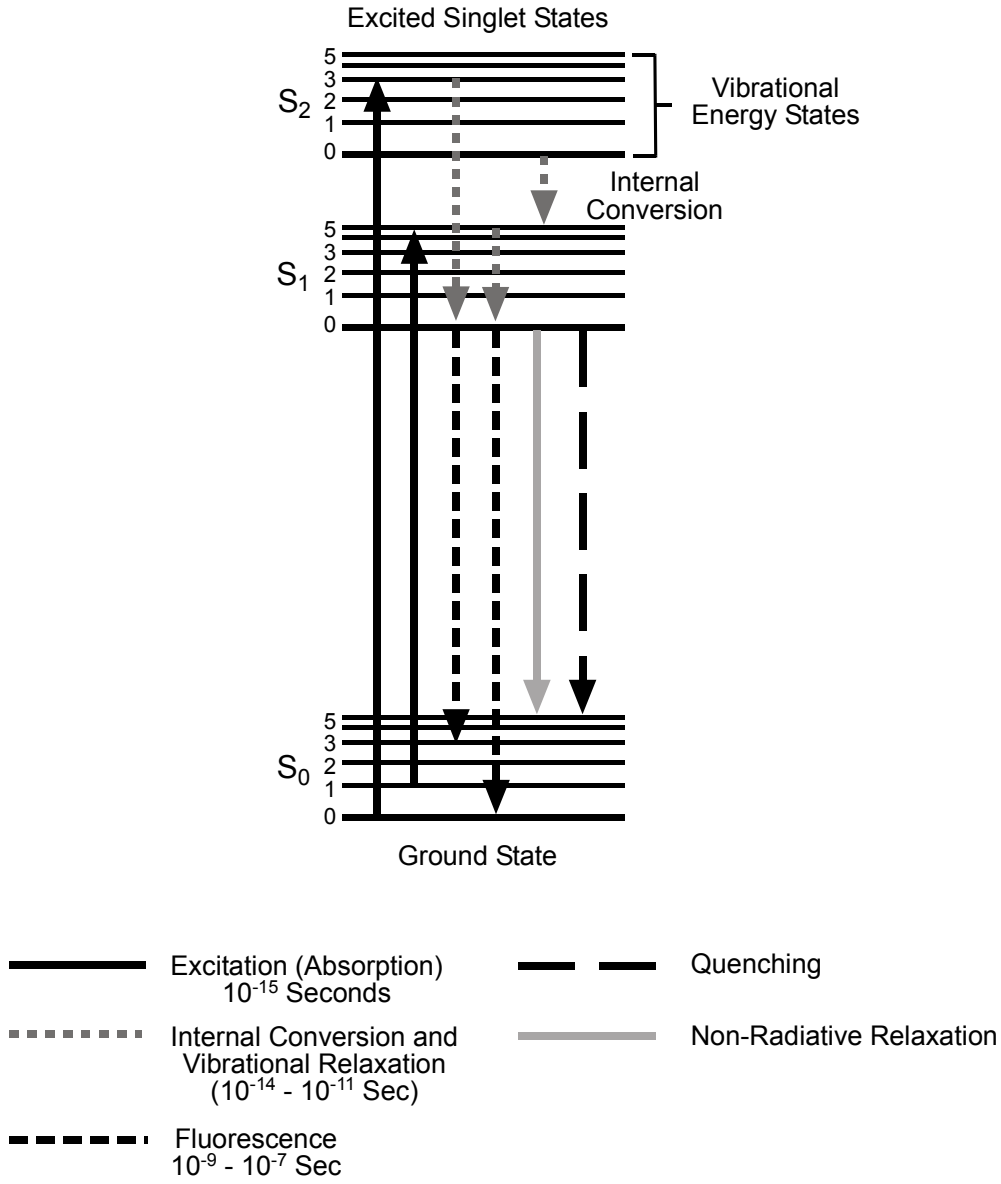


Figure 1.1: Jablonski Diagram illustrating changing energy states of electrons "excited" during the process of fluorescence

DOM source and character have been extensively investigated using fluorescence spectroscopy (Coble, 1996; Mounier et al., 1999; Hautala et al., 2000; Parlanti et al., 2000; Clark et al., 2002; Katsuyama and Nobuhito, 2002;

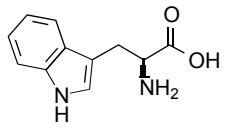
Her et al., 2003; Yamashita and Tanoue, 2003; Cammack et al., 2004; Jaffe et al., 2004; Cory et al., 2007; Lapierre and Frenette., 2009) as have its interactions and alteration through as a result of various physical and chemical processes (as cited in 1.4) all of which vary on both spatial (Galapate et al., 1998; Del Castillo et al., 1999; Clark et al., 2002; Maurice et al., 2002; Baker et al., 2003; Baker and Spencer, 2004; Del Vecchio and Blough, 2004a; Brooks et al., 2005; Jaffe et al., 2008; Mladenov et al., 2008; Bieroza et al., 2009) and temporal (Baker, 2002a; 2002b; Baker et al., 2003; Brooks et al., 2005; Jaffe et al., 2008, Mladenov et al., 2008; Bieroza et al., 2009) scales.

Fluorescence studies in the aquatic environment centre on the complex mixture of ubiquitous, heterogeneous compounds (Westerhoff et al., 2001; Her et al., 2003) which comprise the fluorescent fractions of DOM. The most commonly studied fluorescent organic components of natural waters include humic substances, derived from the break-down of plant material by biological and chemical processes in the terrestrial and aquatic environments (Elkins and Nelson, 2001; Stedmon et al., 2003; Patel-Sorrentino et al., 2004) and amino acids in proteins and peptides. Humic substances can be sub-divided into three categories, chemically defined by solubility at different pH.

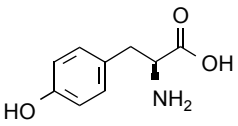
Humic acids are insoluble in aqueous solution at pH lower than 2, but soluble at higher pH. *Fulvic acids* are soluble in water under all pH conditions. *Humins* are insoluble in water under any pH conditions. (Aiken et al.,1985).

Three fluorescent amino acids (tryptophan, tyrosine and phenylalanine) are indicative of proteins and peptides. The fluorescence of these specific amino acids is due to the presence of an indole group (a fused ring heterocycle containing both a benzene ring and a heterocyclic aromatic ring in which a nitrogen atom occurs as part of a ring) or some other aromatic ring structure in which electrons are “shared” rather than occurring as opposite spin pairs and are therefore loosely held and available for promotion to higher energy level. Debate continues about the origin of protein-like fluorescence, whether it is entirely from free amino acids in the DOM pool (Yamashita and Tanoue, 2003), or partially from aromatic amino acids bound in proteins or organism cell walls (Determann et al., 1998). There is clear evidence for a bacterial origin. Shelley et al., (1980a, 1980b) and Dalterio et al., (1986) proposed identification of bacteria by their fluorescence characteristics. Determann et al., (1998) postulated that it may be possible to identify algal and bacterial tryptophan by emission wavelength, compared with that of tryptophan standard. Cammack et al., (2004) and Elliott et al., (2006), found that tryptophan-like fluorescence relates to the activity of a viable bacterial community being both a biological product of that community and a bioavailable substrate. Figure 1.2 demonstrates the structures of tryptophan, tyrosine and phenylalanine standards and theoretical humic and fulvic acids.

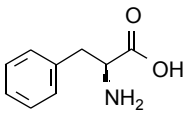
Structure of tryptophan, tyrosine, phenylalanine



trp w Tryptophan



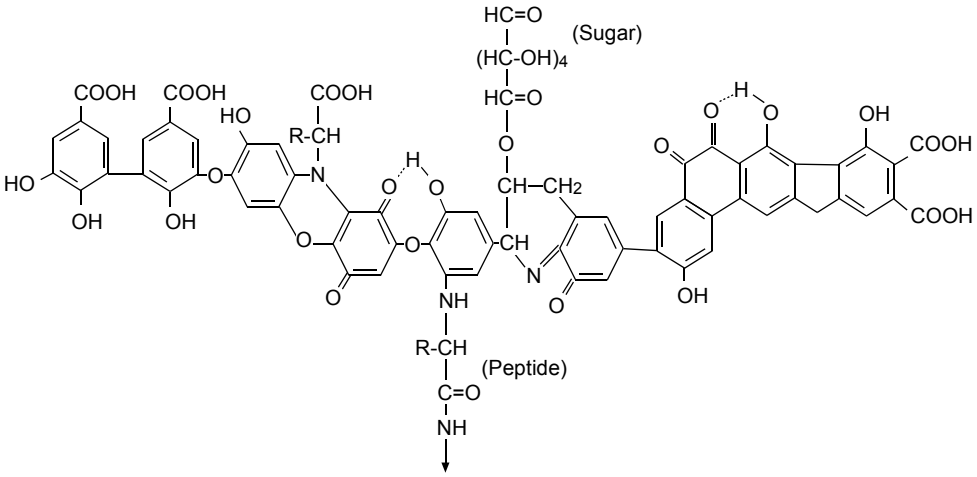
tyr y Tyrosin



phe f Phenylalanin

Theoretical humic acid

Stevenson, (1982) cited in Aitken et al., (1985)



Theoretical fulvic acid

Buffle, (1977) cited in Aitken et al., (1985)

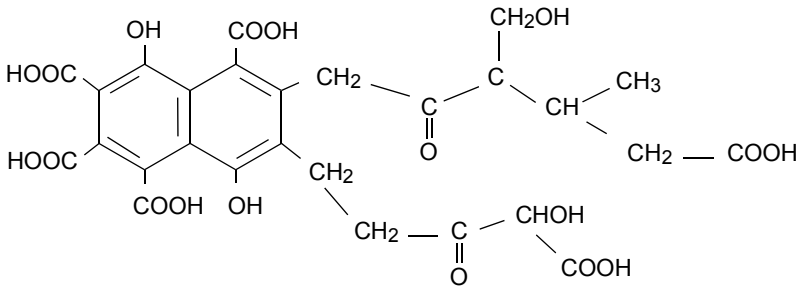


Figure 1.2: Pictorial representation of actual and hypothetical structures of common aquatic fluorophores

Due to the difficulties associated with definitively identifying individual fluorescent compounds in waters these groups of fluorophores are commonly named humic-like, fulvic-like and protein-like (specifically tryptophan- or tyrosine-like), so called because their fluorescence occurs in the same area of optical space as standards of these materials. However, fluorescence in the natural environment may be different to that observed in the laboratory under controlled conditions, using model compounds. Environmental protein fluorescence resembles that of amino acids, as observed in laboratory analysis of amino acid standards (Mayer et al., 1999), but with a blue shift to shorter emission wavelengths due to differences in the behaviour of amino acids in the different microenvironments present within proteins (Determann et al., 1998). International Humic Substances Society (IHSS) samples have been used as standards for the humic and fulvic fractions (Senesi et al., 1989; Fu et al., 2006) and while they provide valuable data, IHSS samples have been isolated and concentrated. These processes may change the structure of the organic material present through preferential removal of groups with certain ionic character, or destruction of functional groups with implications on the behaviour and character of the standards compared to unaltered organic matter in natural water samples. The fluorescence of humic and fulvic acids from a range of sources are examined in detail by Senesi et al., (1991) who determined that the molecular components of humic and fulvic acids differ with source and impart a specific spectral signature.

Table 1.1 lists the various naming formats of common aquatic fluorophores found in literature. This review will use the Coble, (1996) names, as illustrated in Figure 1.3.

Table 1.1: Naming formats of common aquatic fluorophore including those of Coble (1996), Parlanti et al., (2000) and Marhaba and Lippincott (2000)

Fluorophore name by author:					
(Coble, 1996)	***(Parlanti et al., 2000)***	***(Marhaba and Lipincott, 2000)***	***Fluorophore type***	***Peak Ex/ Em wavelength (nm)***	***Author***
A	α'	*Hydrophobic acid (HPOA)*	*Humic-like*	*237-260/400-500*	
C	α		*Humic-like*	*300-370/400-500*	
M	β		*Marine Humic-like*	*312/380-420*	
B	γ	*Hydrophobic neutral (HPON)*	*Tyrosine-like*	*225-237/309-321 and 275/310*	
T	δ	*Hydrophobic base (HPOB) Hydrophilic acid (HPIA) Hydrophilic neutral (HPIN)*	*Tryptophan-like*	*225-237/340-381 and 275/340*	
			Chlorophyll a	*431/670*	*(Moberg et al., 2001)*
			Chlorophyll b	435/659	*(Moberg et al., 2001)*
			Napthalene	*220-230/340-370*	*(Baker and Curry, 2004)*
			Fluorescent whitening agent	*260/430 260/540 400/ 460*	*(Westerhoff et al., 2001)*

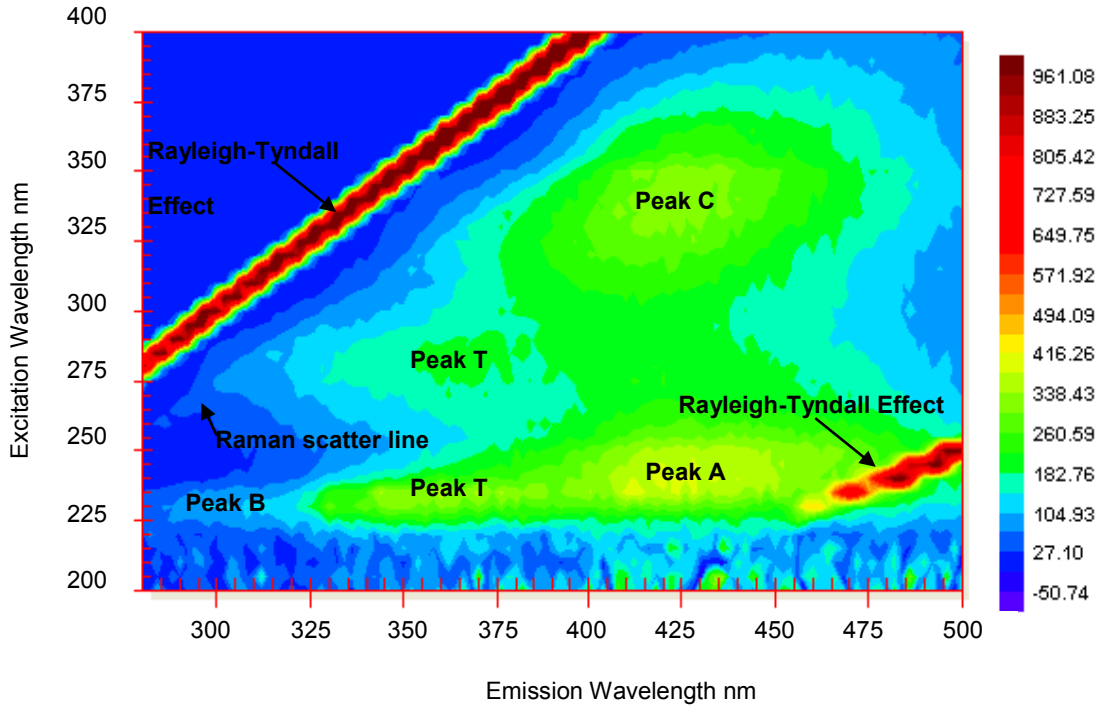


Figure 1.3: EEM showing common EEM features (on an EEM derived for a sewage effluent) and the position of peaks A, C, B and T as named by Coble, (1996)

Another method of identifying fluorophores is by their chemical character after fractionation (Wu et al., 1993; Hautala et al., 2000; Marhaba and Lippincott, 2000) - hydrophobic or hydrophilic and acid, base or neutral. This naming system is less common than the Coble, (1996) (A, C, B, T) or standard nomenclature (e.g. humic-like). It is, however, used in studies in which separation by retention on ion-exchange columns are undertaken. Any information provided by fluorescence spectroscopy about molecular size, aromacity or aliphatic properties allows additional chemical composition classification of the DOM, and better understanding of its bioavailability and reactions in natural waters.

1.3. Fluorescence Spectroscopic Techniques

Improvements in technology, particularly light-source wavelength range and stability, scanning speed and data processing capability, have enabled fluorescence spectroscopy to become a more flexible, rapid and portable diagnostic tool. It is possible, using simple equipment, to target a single excitation and emission wavelength pair, diagnostic of a specific molecule (Skoog et al., 1996; Petrenko et al., 1997; Determann et al., 1998; Chen, 1999; Thoss et al., 2000; Clark et al., 2002; Nagao et al., 2002; Del Vecchio and Blough, 2004a) which is useful in determining the presence or absence and character of a target compound. However, the technique is relatively slow and inflexible, particularly if a range of excitation and emission wavelength scans are required for the study of more than one fluorophore.

Other available techniques include fluorescence emission spectrometry, in which emission is scanned over a range of wavelengths for a fixed excitation wavelength (Ferrari et al., 1996; Hautala et al., 2000). This increases the range of fluorophores that might be found, but output is restricted to a linear scan, in which the choice of excitation wavelength determines the molecules that may be identified. Synchronous Fluorescence Scanning (SFS) has similar drawbacks. Despite scanning both excitation and emission wavelengths it is still a linear technique. Emission wavelength is measured at an offset from the excitation wavelength, commonly by 12-60nm (Senesi et al., 1989; Senesi et al., 1991; Yang and Zhang, 1995; Pullin and Cabanas, 1997; Galapate et al., 1998; Lombardi and Jardim, 1999; Kalbitz et al., 2000; Marhaba and

Lippincott, 2000; Piana and Zahir, 2000; Kalbitz and Geyer, 2001; Westerhoff et al., 2001; Reynolds, 2003; Bengraine and Marhaba, 2004; Jaffe et al., 2004).

Today, excitation emission matrix fluorescence spectroscopy (EEMS) is the state-of-the-art technique used. It was not until the mid 1990s (Coble, 1996) that EEMS became common in aquatic studies. The principal of EEMS is that excitation, emission and fluorescence intensity can be scanned over a range of wavelengths synchronously and plotted on a single chart, developing a "map" of optical space, an Excitation Emission Matrix (EEM). Examples of such "maps" can be seen in Figure 1.4, which include typical EEMs of International Humic Substance Standards (IHSS) of a Suwannee River humic and fulvic acid and a tryptophan and tyrosine standard, at appropriate concentrations for obtaining a clear EEM. Figure 1.5 includes a typical EEM of marine, surface water, untreated and treated sewage at appropriate dilutions.

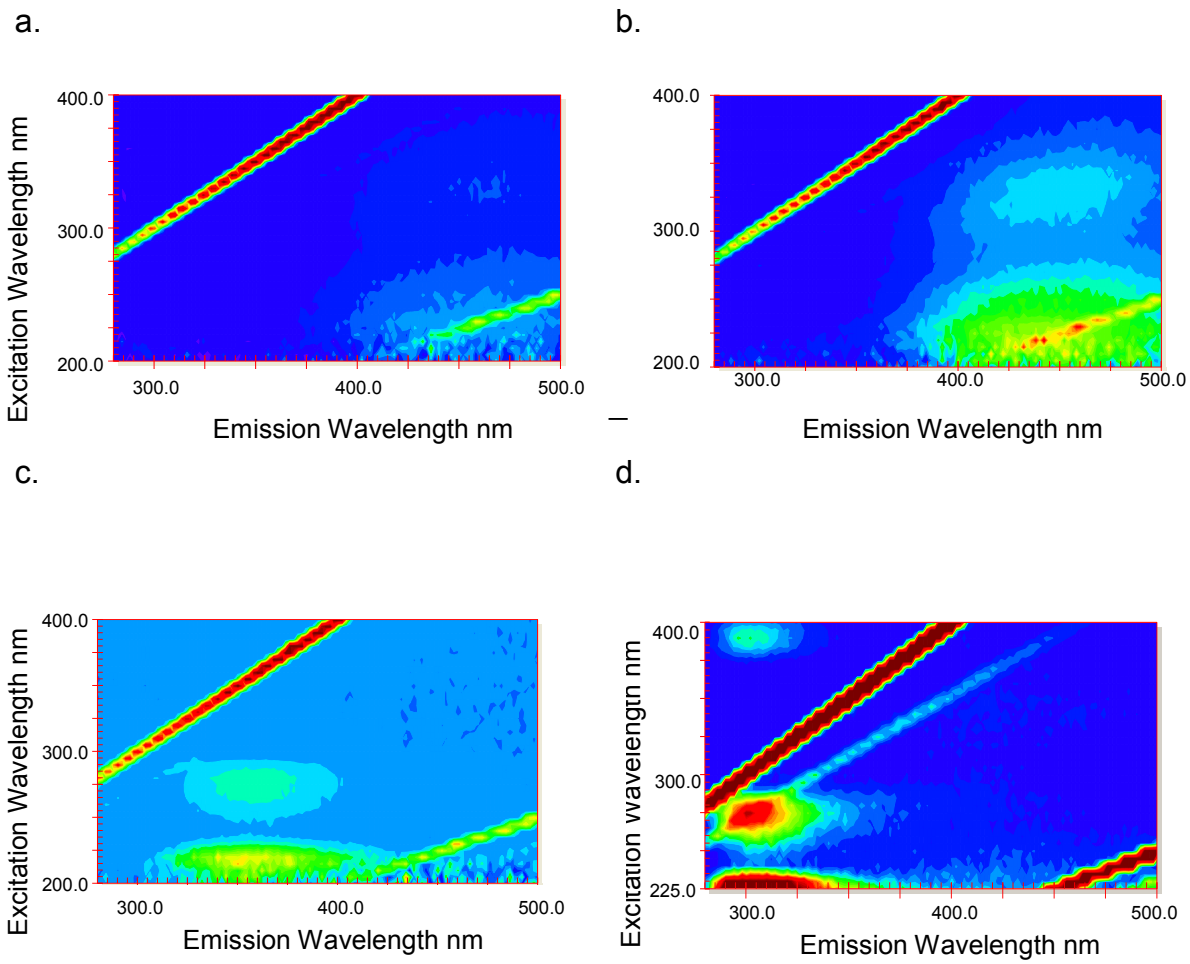


Figure 1.4: Fluorescence EEMs of common standards a). humic acid standard 12.5ppm, b). fulvic acid standard 12.5ppm, c). tryptophan standard 0.1ppm and d) tyrosine standard 0.2ppm (measured at enhanced sensitivity) provided by C. Muller (2007).

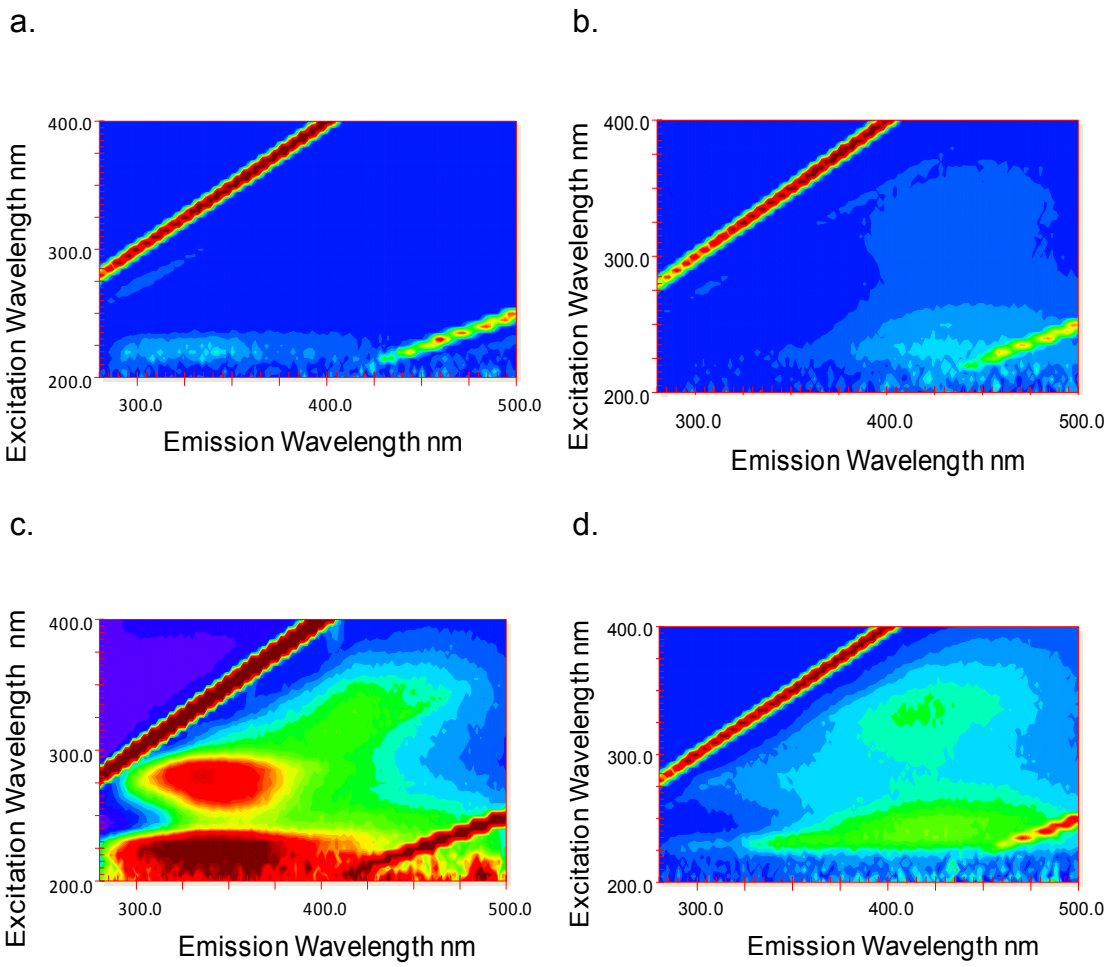


Figure 1.5: Typical EEMs of a number of natural waters a) marine, b) surface water, c) untreated wastewater and d) treated effluent at appropriate dilutions. Fluorescence intensity scale as shown in Figure 1.3

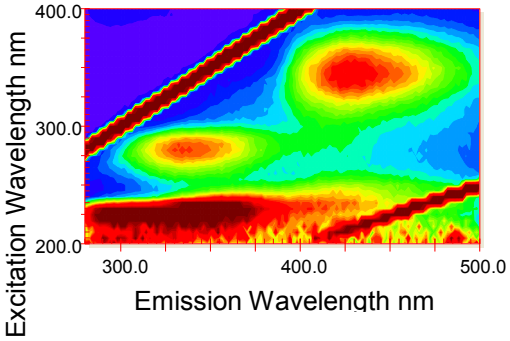
EEMS is rapid (~1 min per sample (Baker, 2001). The production of a 3-D plot of fluorescence excitation wavelength, emission wavelength and intensity allows the visualisation of a range of fluorophores in a given sample, in their relative positions in optical space. Relative concentrations can be determined based upon calibration of fluorescence intensity against TOC (Baker, 2002c) or standards with detection limits at ppb or ppm levels depending on the fluorophore. The technique is non-destructive and requires little or no sample preparation. An additional feature of the EEMS approach is

the vast array of data available for interpretation within an EEM (Lombardi and Jardim, 1999). Attempts have been made to extend the understanding of fluorophore character and concentration by utilising this large amount of data, for example by the analysis of the shape under the peak, and the use of statistical techniques such as “Analysis of Variance” (ANOVA) (Bertilsson et al., 2004; Jaffe et al., 2004; Smith et al., 2004), “Parallel Factor Analysis” (PARAFAC) (Moberg et al., 2001; Brunsdon and Baker, 2002; Stedmon et al., 2003; Olivieri et al., 2004) and “Partial Least Squares regression” (PLS) (Ferrer et al., 1998; Vassel and Praet, 2002; Bengraine and Marhaba, 2004) to analyse both individual and groups of EEM.

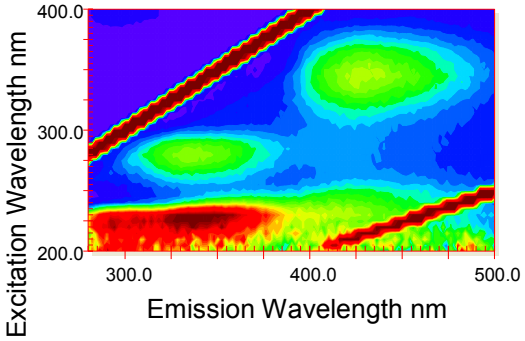
Fluorescence analyses of DOM have the potential to be constrained by a lack of understanding of the effects of inner filtering, the absorption and re-emission of emitted energy at a longer wavelength by surrounding molecules. This is particularly an issue in concentrated solutions. Inner filtering has been identified as a shift to a longer emission wavelength (red shift) in known fluorophores in model and natural solutions due to the concentration of fluorophores in the solution (Yang and Zhang, 1995; Mobed et al., 1996) and a shift to a shorter emission wavelength (blue-shift) was observed with decreasing solution concentration (Hautala et al., 2000). There is a disagreement in the literature as to the concentration of DOM at which inner-filter effects interfere. Various suggestions have been made about the optimum concentration for DOM analysis in water to minimise the inner filtering effect, from 1mgl^{-1} (Hautala et al., 2000; Westerhoff et al., 2001) to 15mgl^{-1} (Yang and Zhang, 1995) and 100mgl^{-1} (Senesi et al., 1991). Vodacek

and Philpot (1987) suggested that concentration effects should be negligible in natural waters as DOC concentration rarely exceeds 20mgl^{-1}. However, high concentration solutions, such as untreated sewage, require dilution prior to fluorescence analysis (Mobed et al., 1996; Kalbitz and Geyer, 2001; Baker et al., 2004). This reduces inner filtering by reducing the concentration of fluorophores in the sample while retaining the relative proportions in solution. The potential for absorption of emitted light by surrounding fluorophores is reduced with the reduction in fluorophores present, so emission wavelength becomes a direct value with no associated energy loss to surrounding molecules. Figure 1.6 illustrates the change in character of EEMs with dilution. Figure 1.7 shows that there is a wide variation in sample fluorescence per carbon, due to variations in DOM character, explaining the lack of a unique total organic carbon (TOC) concentration at which inner filtering is found to be negated (in surface water and effluent samples from Southwest England).

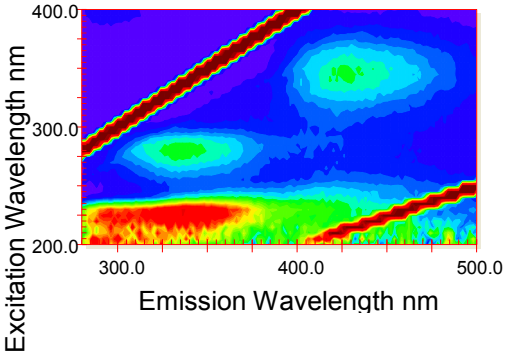
100% concentration



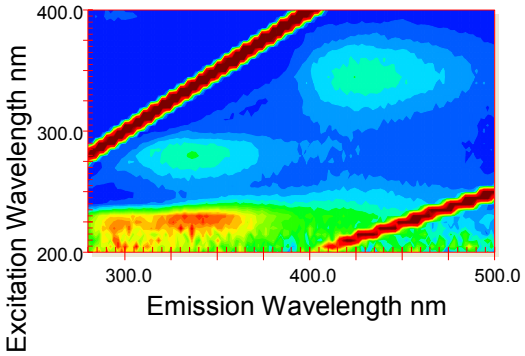
50% concentration



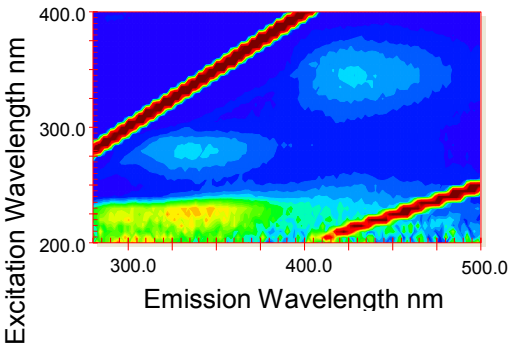
25% concentration



12.5% concentration



6.25% concentration



3.125% concentration

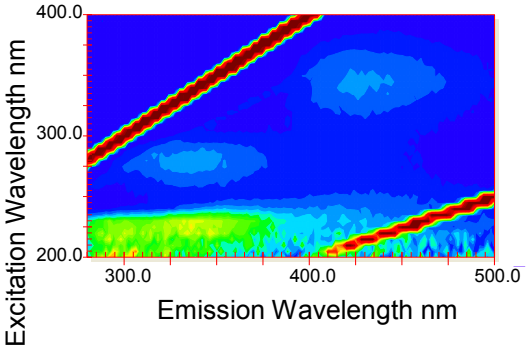


Figure 1.6: Sewage effluent dilution series (sample 725823) by EEM demonstrating the change in EEM character with dilution. Figure 1.6 illustrates the change in character of EEMs with dilution. Fluorescence intensity scale as shown in Figure 1.3. Initial TOC value of undiluted sample = 20.69mgl^{-1}.

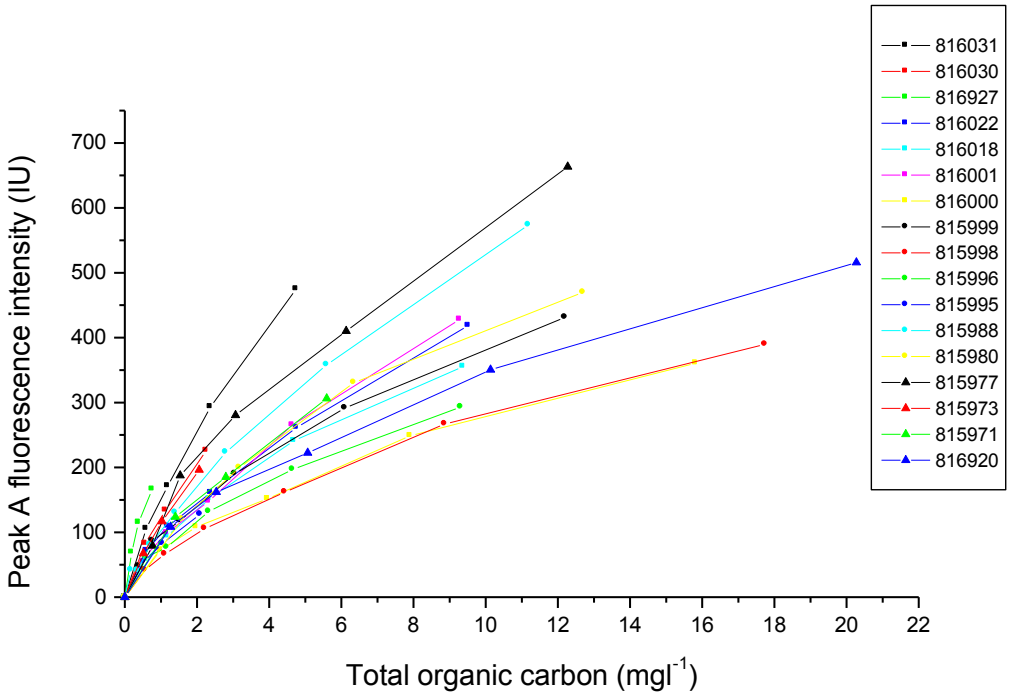


Figure 1.7: Variation in sample fluorescence per carbon as a result of varying DOM character illustrated by plot of Peak A fluorescence intensity change with dilution. This explains the lack of a unique TOC concentration at which inner filtering is negated in surface water and effluent samples from South West England. Samples are identified by reference numbers and are from the following (EA named) locations: 816031 (Mincinglake at Calthorpe Road), 816030 (Alphin Brook at Alphinton Footbridge), 816927 (Alphin Brook at Countess Weir Bridge), 816022 (Dawlish STW UV disinfection), 816018 (Exeter Countess Weir STW), 816001 (C.A.R.E Community East Anstey), 816000 (South Molton STW), 815999 (North Molton STW), 815998 Bishops Nympton STW), 815996 (Wrington STW), 815995 (Congresby Yeo Beam B), 815988 (Wick St Lawrence STW), 815980 (Pucksey Brook, Broadwey), 815977 (Miscellaneous Okement), 815973 (Mill Stream at Cowards Lake), 815971 (Mill Stream d/s Horsepool), 816920(Ken and Kennford STW).

A further interference, scatter by particulates and larger colloids, is also reduced by dilution again by reduction in concentration. However scatter is only effectively reduced by filtering, which also ensures that only dissolved organic matter contributes to the fluorescence signature.

Finally, linear features evident on fluorescence EEMs are the Raman line and the Rayleigh-Tyndall features as illustrated in Figure 1.3. The Raman line is a faint linear trace at excitation wavelengths between 260nm and 350nm and emission wavelengths 280nm-400nm which is an optical manifestation of the scattering properties of water due to the vibration of molecular O-H covalent bonds with the application of light energy. The position of the Raman line is dependent upon the wavelength of the incident radiation. Studies commonly correct for this effect by gross spectral subtraction. The shorter excitation wavelength forms of tyrosine-like fluorescence (Peak B, Coble, 1996) can be obscured by the position of the Raman line. Normalisation of the fluorescence intensity data with the Raman data provides a useful internal standard. The Rayleigh-Tyndall effect is a visible feature which is source excitation energy reflected off the cuvette walls and which is visible in Figure 1.3 at emission wavelength = emission wavelength and at emission wavelength = 2 x excitation wavelength.

1.4. Environmental Effects on DOM Fluorescence

The wavelengths at which molecules fluoresce and the intensity of the fluorescence does not just depend on the properties of the DOM but can be affected by a number of factors such as changes in pH (Patel-Sorretino et al., 2002), quenching by chelation with metal ions (Reynolds and Ahmad, 1995) and changes in temperature (Baker, 2005).

1.4.1. pH

Changes in observed fluorescence intensity are a result of conformational changes in the molecules (Myneni et al., 1999; Westerhoff et al., 2001) exposing or hiding fluorescent parts of the molecule. Low pH causes the molecule to coil, while raising pH extends the molecule. The degree of pH effect depends upon the fluorophore. Fluorescence spectroscopy has been applied to solutions at a range of pH (2-12) although pH is sometimes altered to near neutral (pH 6-7) prior to fluorescence analysis (Yang and Zhang, 1995; Her et al., 2003). Vodacek and Philpott (1987) identified a trend of increasing fluorescence intensity as pH increased from pH 4 to pH 5.5 in humic substances, above which a continued, but less dramatic, increase was found. Westerhoff et al., (2001) identified a decrease in fluorescence intensity of 30-40% as pH decreased from pH 7 to pH 3 in fulvic acid standards and treated municipal wastewater and suggested an optimum fluorescence analysis pH of 3 for water samples. Reynolds, (2003) determined that in tryptophan standards of pH < 4.5 fluorescence decreased by up to 15%, pH 5-8 there was little impact, and at >pH 8 fluorescence was enhanced by up to 30%. However, Baker et al., (2007) and Spencer et al., (2007) did not identify any significant change in tryptophan-like fluorescence in natural samples with changing pH. It was also found that peak B (tyrosine-like) was more sensitive to pH changes than the other peaks. Patel-Sorrentino et al., (2002) investigated the impact of changing pH on A and C peaks in surface waters and found that fluorescence intensity generally increases with an increase from pH 2 to about pH 12 at which point a minor decrease is observed. It was

also found that changes in fluorescence intensity as a result of pH change are reversible (Vodacek and Philpot, 1987; Patel-Sorrentino et al., 2002). In aquatic systems pH is normally between pH 5-9. Over this range fluorescence intensity of all fluorophores has been found to increase by about 10% although the scale of the increase is fluorophore specific. This is unlikely to have an impact on fluorescence analysis of most natural waters and is less than the variability caused by changes in DOM source or character. The effect of changing pH on fluorescence is reviewed in more detail in Chapter 5.

1.4.2. Metal Ions

In natural waters DOM fluorescence, particularly of humic substances (peaks A and C), are affected by the presence of metals through the formation of organo-metal complexes. Fluorescence may be quenched (Estevez da Silva et al., 1998) or enhanced in certain spectral regions (Blaser et al., 1998; Sharpless and McGown, 1999). Most studies analyse the effect of metals upon humic and fulvic acid fluorescence in controlled laboratory conditions at low concentrations of both, avoiding the creation of insoluble complexes which affect the optical character of the solution (Estevez da Silva et al., 1998; Elkins and Nelson, 2001).

In humic substance standards, fluorescence of fulvic acids is quenched in the presence of excess copper, iron and aluminium by varying degrees at concentration as low as 0.1mgl^{-1} (Vodacek and Philpott, 1987; Estevez da Silva et al., 1998; Ohno et al., 2008). Aluminium and copper quenched

fluorescence intensity by up to 40% in concentrations up to 2mgl^{-1} (Reynolds and Ahmad, 1995). Iron quenches more efficiently than chromate, lead, copper and nickel at pH 4 and 8 for a range of humic substances (Piana and Zahir, 2000). Terrestrial humic acids were found to show a general decrease in fluorescence intensity, which could be a result of self-quenching in larger aggregates (Sharpless and McGown, 1999) while aquatic humic acids demonstrate enhancement in some regions of the spectra (Sharpless and McGown, 1999; Elkins and Nelson, 2001) with a related red-shift in excitation wavelength and blue-shift in emission wavelength. The wavelength changes are related to changes in the structure of the humic substances particularly on addition of aluminium (Elkins and Nelson, 2001). However, the change in molecular conformation is likely to be specific to the water type and metal added as Wu et al., (2004) identified a red-shift to longer excitation and emission wavelengths in surface waters upon the addition of mercury.

1.4.3. Photodegradation

The study of photodegradation, photobleaching or photo-oxidation of DOM is usually related to the creation of bioavailable substrate from DOM in aquatic environments, characterisation of the optical and physical properties of humic substances and seasonal and temporal changes in these characteristics. The effects of photochemical degradation on DOM have been studied using fluorescence techniques in a range of aquatic environments. The effects of photo-oxidation of organic matter are studied through analysis of changes in intrinsic fluorescence intensity (Skoog et al., 1996; Moran et al., 2000; Del

Vecchio and Blough, 2002; Bertilsson et al., 2004) or peak position (Moran et al., 2000; Boehme et al., 2004).

Irradiation methodology is split between the use of high output artificial light filtered to provide the required wavelengths (Gao and Zepp, 1998; Moran et al., 2000; Del Vecchio and Blough, 2002b; Miller et al., 2002; Uyguner and Bekbolet, 2005), natural sunlight (Amon and Benner, 1996; Skoog et al., 1996; Bertilsson et al., 2004) and in situ analysis (Skoog et al., 1996),

DOM may be degraded by direct or indirect means (direct alteration of the DOM structure or indirect chemical changes through reactions with free radicals created by the application of light) (Amon and Benner, 1996). The process is enhanced by the presence of iron and oxygen through photo-fenton reactions (light induced reactions catalysed by the presence of iron and oxygen) (Fukushima et al., 2001; White et al., 2003) while the presence of copper may inhibit the formation of some photolytic products.

There is general consensus between existing studies regarding the effect of photochemical reactions upon natural waters which are reviewed in greater detail by Benner and Ziegler, (1999) with no comment on the use of fluorescence in analysis. In general a decrease in UV- absorbance (Ferrari et al.,1996), fluorescence intensity (Moran et al., 2000; White et al., 2003; Waiser and Robarts, 2004; Uyguner and Bekbolet, 2005), aromatic character (Benner and Ziegler, 1999), molecular size (Benner and Ziegler, 1999; Fukushima et al., 2001; Uyguner and Bekbolet, 2005; Lou and Xie, 2006) and

DOC is seen (Moran et al., 2000; Clark et al., 2002). Photochemical products include dissolved inorganic carbon (DIC) (Benner and Ziegler, 1999), carbohydrates (Uyguner and Bekbolet, 2005) and volatile organic carbon compounds (Clark et al., 2002). Results are inconsistent regarding increasing bioavailability of photochemical products with Bertilsson et al., (2004) demonstrating an apparent balance in increasing DOM bioavailability and bacterial DOM production, with no net gain or loss of DOM.

The extent of photodegradation of DOM is related to the structure (Benner and Ziegler, 1999) and source (Skoog et al., 1996; Judd et al., 2006; Vione et al., 2009) of the DOM and the incidence of irradiation. Water from depth and waters with greater terrestrial input (Skoog et al., 1996) have a greater potential for photodegradation than surface waters. This may be due to the prior degradation of surface water DOM by UV exposure which is difficult to quantify, and the composition of the terrestrial organic matter. Other work concludes that shallow waters e.g. wetlands, coastal shelf regions (Del Vecchio and Blough, 2004a; Waiser and Robarts, 2004) show high DOM photochemical alteration as a result of greater UV-B penetration, mixing within the water body and input of terrestrial material. Humic-like compounds (particularly peak C) are found to be more likely to degrade than peaks A or T (Moran et al., 2000) and no work, other than Moran et al., (2000), has been found relating to the photodegradation of the B or T peaks. In natural waters photodegradation is found to have an impact on DOM structure and character with a change to smaller molecules, an associated effect on bioavailability and

so is likely to change the fluorescence character by the presence or absence of peaks or changes in their relative intensities.

The effect of light exposure upon aquatic organic matter fluorescence is reviewed in detail in Chapter 5.

1.4.4. Temperature

Early work by Wehry, (1973) cited in Vodacek and Philpot, (1987) states that fluorescence is inversely related to temperature due to increased collisional quenching at higher temperatures. Fluorescence changes caused by temperature should be reversible as no change is made to the structure of the DOM. However experiments showed that non-reversible changes did occur, possibly as a result of the application of a light-source, which may be due to photodegradation or thermal decomposition (Vodacek and Philpott, 1987). Conventionally, temperature has been held constant during fluorescence analysis to avoid any interference from thermal quenching, although more recent works (Baker, 2005; Seredynska-Sobecka et al., 2007) have investigated the relative thermal quenching properties of the fluorophores present in DOM as a technique to probe DOM structure.

In surface waters the effect of freezing has been studied relative to organic matter fluorescence characteristics (Spencer et al., 2007; Hudson et al., 2009). Fluorescence intensity of all peaks was found to decrease with cycles of freezing and thawing (Hudson et al., 2009) although the degree of change

was sample specific. Moves to shorter excitation and emission wavelengths (blue shifts) have also been identified as a result of freezing (Spencer et al., 2007) although again, results were highly variable. The effect of freezing on organic matter fluorescence is reviewed more fully in Chapter 6.

1.5. Applications of DOM Fluorescence in Natural Waters

1.5.1. Marine and Estuarine DOM

Since initial work by Kalle, (1949) cited in Coble, (1996) tracing riverine dissolved organic carbon (DOC) in the ocean using fluorescence, fluorescence spectroscopy has been used increasingly in the study of DOM in marine and estuarine waters. Common applications include study of the fluorescence properties of DOM and CDOM (Ferrari et al., 1996; Mopper at al., 1996; Del Castillo et al., 1999; Lombardi and Jardim, 1999) as a tool for determining biological activity and associated protein fluorescence (Determann et al., 1998; Mayer et al., 1999; Parlanti et al., 2000; Yamashita and Tanoue, 2003; Jaffe et al., 2004); characterisation of DOM from different sources (Coble, 1996; Clark et al., 2002; Jaffe et al., 2004; Murphy et al., 2008 (in ballast waters)); fluorescence of organics held in, and released from, sediment (Komada et al., 2002) and mixing of water bodies (de Souza Sierra et al., 1997; Klinkhammer et al., 2000).

Marine waters are rich in the marine humic peak (M) with intense tryptophan-like fluorescence (T) related to biological activity particularly in areas of high primary productivity i.e. surface waters and areas of upwelling. The marine

humic fluorophore (M) was initially thought to be either an independent marine entity or an alteration product of terrestrial humic-like material C (Coble, 1996) (Table 1). Further work by Parlanti et al., (2000) considered the importance of fluorophore β, which corresponded with Coble's marine humic (M) and which was associated with biological activity and elevated protein concentrations in areas of high primary productivity (Table 1.1). This suggested that it is not an alteration product of the terrestrial originated C humic-like material, but a marine derived humic-like compound constituting "fresh" marine humic material, prevalent in surface waters with a direct correlation with biological activity and salinity (Coble, 1996; Parlanti et al., 2000).

A change in humic substance character is commonly observed with depth and distance from shore. C-type fluorescence intensity is seen to decrease with an increase in marine influence (Del Castillo et al., 1999; Clark et al., 2002). Terrestrial type humic material is present in rivers and deep marine waters suggesting that this may represent older, more degraded and humified material (Komada et al., 2002).

Peaks T and B (protein-like fluorescence) in marine and estuarine environments are a result of a mixture of autochthonous (created in situ) and allochthonous (created elsewhere and transported) sources. Tryptophan-like fluorescence (peak T) is common in waters subject to anthropogenic influence such as bays, estuaries, coastal areas, also areas of high primary productivity and pore waters (Coble, 1996) and so is thought to derive directly from bacterial activity (Yamashita and Tanoue, 2003; Cammack et al., 2004).

Tyrosine-like fluorescence (peak B) is present in all marine waters at all depths (Mayer et al., 1999; Yamashita and Tanoue, 2003). Peaks T and B represent either intrinsically fluorescent molecules which constitute bioavailable organic fractions of DOM or fluorescent products of microbial activity, existing on the bioavailable, labile organic fractions, or perhaps a mixture of both.

1.5.2. Surface Water DOM Fluorescence

The main applications of fluorescence spectroscopy to surface waters have been in the determination of the optical properties of DOM (Battin,1998); the influence of pH on fluorescence of organic matter (Patel-Sorrentino et al., 2002); characterisation of DOM composition and source (Mounier et al.,1999; Hautala et al., 2000; Katsuyama and Nobuhito, 2002; Her et al., 2003); comparison of organic matter fluorescence with IHSS model compounds (Senesi et al., 1989; Wu et al., 1993; Kalbitz and Geyer, 2001) and determination of water source by fluorescence fingerprinting (Thoss et al., 2000; Yan et al., 2000; Newson et al., 2001; Baker, 2002c). However, the study of organic matter fluorescence in the surface water environment is not yet as widespread as that in the marine sciences.

Naturally occurring, fluorescent, surface water DOM is predominantly composed of humic (C and A) fractions from the breakdown of organic material in water, riparian zones (Katsuyama and Nobuhito, 2002) and other soils. Increasing urbanisation is observed through the presence of peak T

(tryptophan-like fluorescent material) with minor peak-B (tyrosine-like material) (see section 1.5.4).

Surface water fluorescence has been investigated with an emphasis on spatial variation (Galapate et al., 1998; Katsuyama and Nobuhito, 2002, Baker et al., 2003; Baker and Spencer, 2004). Baker and Spencer (2004) identified a change from natural, humic-rich waters in upland regions (peaks A and C) to "peak-T"-rich waters downstream with increasing anthropogenic input and urbanisation. Similar patterns were observed on a catchment scale, relating changes in fluorescence spectrum to upstream and downstream character and known anthropogenic inputs e.g. sewage treatment effluents, combined sewer overflows (CSOs), airport wash off (Baker et al., 2003).

Organic matter in rivers also shows seasonally differential fluorescence intensity. Peak T, associated with CSO discharges, is observed to be more intense in summer, probably due to reduced base flows and lower dilution (Baker, 2002a; Baker et al., 2003). Organic matter character in rivers shows clear seasonal trends due to flow rate and the impact of wetting and drying on surrounding land (Katsuyama and Nobuhito, 2002).

1.5.3. Wastewater Fluorescence

Crude sewage is composed of heterogeneous mixture of compounds including fulvic acids, proteins, carbohydrates and lipids with varying contributions from organic surfactants, nucleic acids and volatile fatty acids

(Ahmad and Reynolds, 1995). It is a mixture of domestic waste, industrial (consented and unconsented) discharges and a domestic element from industrial premises (kitchens and toilets), in addition to surface runoff and storm flow. Composition varies depending upon the age and type of sewerage system in the catchment (separate or combined), time of day (Reynolds and Ahmad, 1997), prevailing and prior weather conditions and type of incoming sewer (gravity or pumped)

In line with the work carried out over the last 20 years in the field of dissolved organic matter (DOM) analysis in natural waters, research has been undertaken into the use of fluorescence as a tool for water treatment process optimisation, water quality assessment and pollution monitoring. These applications have important ramifications for the water industry and environmental regulators. By monitoring DOM levels through a treatment works the operation of the works may be optimised, and up to 40% of energy costs could be saved by optimising process efficiency (particularly aeration) (Ahmad and Reynolds, 1999). Field-based fluorescence determination of water quality would allow rapid assessment of the organic matter content and potential polluting load of a discharge or water-body, the identification and tracking of pollution incidents and therefore more rapid remedial action could be taken, avoiding statutory fines.

Studies of EEMs of untreated wastewaters show that they commonly comprise a broad humic-type peak C with intense T peaks and B peaks which occur at the same position in optical space as standard solutions of

tryptophan and tyrosine respectively (Baker et al., 2004). Also present are peak A and, occasionally, other peaks though to be related to the presence of fluorescent whitening agents (FWAs) from detergents (Westerhoff et al., 2001).

Figure 1.8 illustrates the difference in character between river waters and sewage effluents from Southwest England in relation to peak C fluorescence and total organic carbon (TOC) concentration with surface waters (freshwater) generally exhibiting lower peak C fluorescence intensity and TOC concentration.

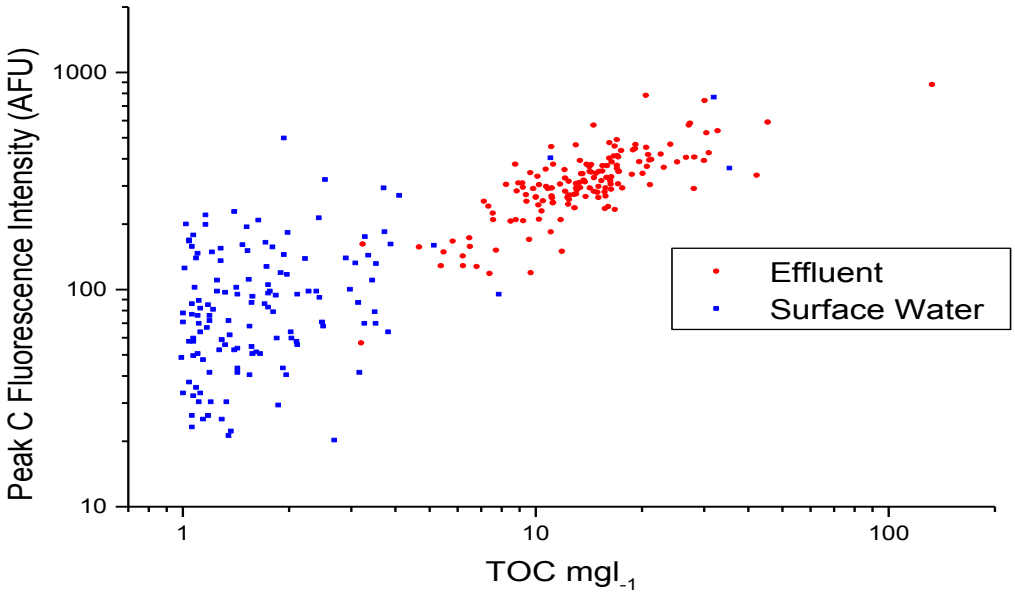


Figure 1.8: Plot showing peak C fluorescence intensity against TOC on a log/log scale for a number of surface waters and effluents from Southwest England with surface waters generally exhibiting lower peak C fluorescence intensity and TOC concentration.

Peak T generally contributes the highest intensity peaks in wastewaters (Reynolds and Ahmad, 1997). Galapate et al. (1998), Baker et al. (2003; 2004) and Reynolds (2003) showed that peak T can be considered as a tracer and relic of anthropogenic material in natural waters due to its peak intensity, even in treated effluents, compared with the background levels of the natural water (see section 5.4.). A number of papers by Ahmad and Reynolds (Ahmad and Reynolds, 1995; 1999; Reynolds and Ahmad, 1997; Reynolds, 2002) have determined that a clear decline in fluorescence intensity of peak T is observed from influent to effluent across a treatment process. Peak T at $\lambda_{ex/em}$ (excitation/ emission wavelengths) 280/340nm was identified as being most likely to relate to the biodegradable fraction of wastewater with up to 90% reduction observed across a treatment works. The work of Cammack et al. (2004) and Elliott et al. (2006) showed peak T fluorescence to be directly associated with the growth stage of bacterial communities. For this reason it is believed that peak T is a more labile fraction of DOM which is preferentially degraded through the treatment process and that it may be used as an indicator of the concentration of biodegradable organic matter in water (Hudson et al., 2008), correlating well with the current measurement of biodegradable organic load (Biochemical Oxygen Demand Test), see Chapter 3.

1.5.4. Fluorescent DOM in Polluted Waters

A number of studies have applied fluorescence spectroscopy to the tracking and characterisation of wastewater in rivers using the fluorescence signature,

particularly of peak T which is associated with readily biodegradable material (see section 1.5.3.) e.g. in Japanese rivers (Galapate et al., 1998) and Japanese lakes (Reynolds, 2003), and British rivers (Baker et al., 2003; Baker et al., 2004) in which EEMs and field-based fluorescence were used, to characterise the waters of the Ouseburn, an urban river in North East England. Seasonal variations in inputs from storm overflows were identified, as were failed CSOs and cross-connected sewers. Field-based fluorescence has also been used to investigate the effect of marine sewage plumes on the biology, optical character and particle-size distribution of coastal waters around an outfall diffuser using peak T fluorescence to track the extent of the plume (Petrenko et al., 1997).

Fluorescent compounds of anthropogenic origin with the potential to enter the surface water system, which may be identified and traced by their fluorescence signature, include fluorescent whitening agents from tissue mills and laundry products (Baker, 2002c), landfill leachate components such as naphthalene (Baker and Curry, 2004), material from agricultural effluents (Baker, 2002b) and treated sewage effluents and sewer discharges (Galapate et al., 1998; Baker et al., 2003; Her et al., 2003; Reynolds, 2003; Baker et al., 2004) represented by fluorescence peaks B and T. Baker et al., (2004) used fluorescence spectrometry to identify pollution events from CSOs and sewer discharges using a portable, field spectrofluorimeter.

1.6. Summary

Fluorescence spectroscopy using excitation emission matrices (EEMs) is a flexible tool which has potential for wide application in studies of aquatic organic matter. Investigations into the source, character and reactions of marine organic matter have previously been the most common applications, however, the characterisation and quantification of allochthonous and autochthonous organic matter in surface waters becoming more widespread (Hudson et al., 2007).

The origin of “tryptophan-like” or “protein-like” fluorescence is unclear. It is not known whether these fluorescence peaks represent a bioavailable substrate, a product of microbial activity or a mixture of both. In addition the position of tryptophan within proteins requires investigation as this may have an impact on the fluorescence of the molecule. The origin of the bioavailable tryptophan-like fluorescence peak is of interest with relation to current chemical and biological water quality monitoring techniques. The most common of these, the BOD_5 test, has a number of inherent difficulties associated with reliance upon the bacterial community and its sensitivity to environmental conditions. However the biggest inconvenience of the test is the 5 day test period which delays identification of potential pollution events. For this reason I will investigate the relationship between fluorescence and BOD_5 with a view to determining whether fluorescence could be used as a rapid, on-site tool for water quality testing and pollution monitoring. Furthermore I will identify whether relationships exist between fluorescence and other water quality

parameters with a view to determining whether fluorescence may also provide a rapid, on site measurement of other common water quality parameters.

Throughout the body of published data the source and fluorescence response of protein-like peaks T and B are less well understood than humic-like peaks A and C but they have been linked to bacterial activity, sewage treatment process efficiency and therefore organic matter bioavailability. I will present results of my research into changes in organic carbon fluorescence in water under a range of environmental conditions, with an assessment of changes in fluorophore concentration, source and character. In particular I will report differences in response of the protein-like (peaks T_1 and T_2) and humic-like (peaks C and A) groups of peaks, and variations in response of the peaks within those groups. I will relate changes in organic matter fluorescence to growth of bacterial colonies and investigate whether this is the source of developing protein-like fluorescence in this work. The environmental conditions upon which I will focus include regimes of varying temperatures and light exposure, freezing/ thawing and dehydration/ rehydration. All of these conditions are environmentally relevant and may promote changes in fluorescence intensity and peak position, reflecting changes in organic carbon character.

In this work I will investigate the character of surface water and effluent organic matter through the use of fluorescence spectroscopy and excitation-emission-matrices. I will identify likely sources of common fluorophores, their bioavailability and determine whether fluorescence may be used as a

measurement of the polluting potential of organic carbon in waters. I will identify the responses and interactions of common organic fluorophores under different storage conditions including novel research into the impact of freezing/ thawing and dehydration/ rehydration with relation to sample character and organic carbon concentration and I will recommend best practice for minimising sample change prior to analysis.

2. MATERIALS AND METHODS

This work comprises 4 experimental studies which use many of the same analytical techniques. This chapter will describe the analytical techniques and method protocols used in this work.

The four experimental studies in this work are:

1. An investigation into the relationship between organic matter fluorescence and BOD_5 in fresh waters and effluents, undertaken with a view to informing the existing body of research into the potential of fluorescence spectroscopy as a laboratory or field surrogate for the BOD_5 test.
2. An investigation into relationships between fluorescence and other common water quality analyses to determine the character of fluorescent organic matter and the potential of fluorescence spectroscopy for on-site or laboratory rapid analysis of water quality.
3. An investigation into changes in organic matter fluorescence in water with time, under different storage conditions with a view to informing the debate about sample storage prior to analysis, transformations of organic matter in the aquatic environment and investigating possible sources and dynamics of individual fluorophores.
4. An investigation into how the fluorescence intensity of fresh water organic matter changes with cycles of dehydration/ rehydration and freezing/ thawing as a laboratory-scale proxy for these events in the environment and to inform the debate on sample storage and stability.

The common analytical elements of Studies 1, 2, 3 and 4 are excitation-emission-matrix (EEM) fluorescence spectroscopy and total organic carbon (TOC) with the 5 day Biochemical Oxygen Demand (BOD_5) also being measured by the Environment Agency (Study 1, 2, 3 and 4). Supplementary analysis for study 3 is provided by measurement of dissolved oxygen (DO), temperature, pH, conductivity and microbiological cell count using a spread plate method and basic colony forming units (CFU) counts. Studies 2 and 4 include a range of water quality analyses commonly undertaken and reported by the Environment Agency.

The common analytical methods are described below, followed by study specific analytical techniques and finally method protocols for each study will be detailed.

2.1. Common Analytical Techniques

2.1.1. Fluorescence Analysis

An EEM was created for each sample using a Varian Cary Eclipse fluorescence spectrometer. The Cary Eclipse uses a xenon light source which flashes at up to 80 flashes per second at 2-3 microsecond intervals, a single Czerny-Turner monochromator which splits the excitation and emitted light into constituent colours, a range of adjustable filters and two photomultiplier tube detectors (Varian Cary Eclipse online help).

Excitation and emission were scanned simultaneously at wavelengths from 200-400nm and 280-500nm at 5nm and 2nm intervals respectively with a 5nm slit width at 9600 nm/min scan rate and at 20°C (regulated by a Peltier temperature controller). The position (excitation and emission wavelength pair in nm) and maximum fluorescence intensity in arbitrary fluorescence units (AFU) of each peak was identified manually and recorded. No post-manufacturer instrument corrections were applied as these are commonly used to make data comparable between analyses undertaken on different instruments. In this work the same spectrophotometer was used throughout.

The Raman value of water (vibrational effect of excitation of the H-O-H molecules) at excitation wavelength 348nm, derived daily from a manufacturer supplied sealed cell, was used as an internal standard to test for instrument drift and fluorescence intensity results are normalised for this value. The Raman value is seen to increase during the study period in Figure 2.1 (collated by A. Baker (2007) personal communication. Data collected from all laboratory users, SILLA, University of Birmingham) reflecting a change in lamp output. However, as fluorescence intensities are always corrected for the Raman value normalised to 20 this change in instrument performance is compensated for in the results presented.

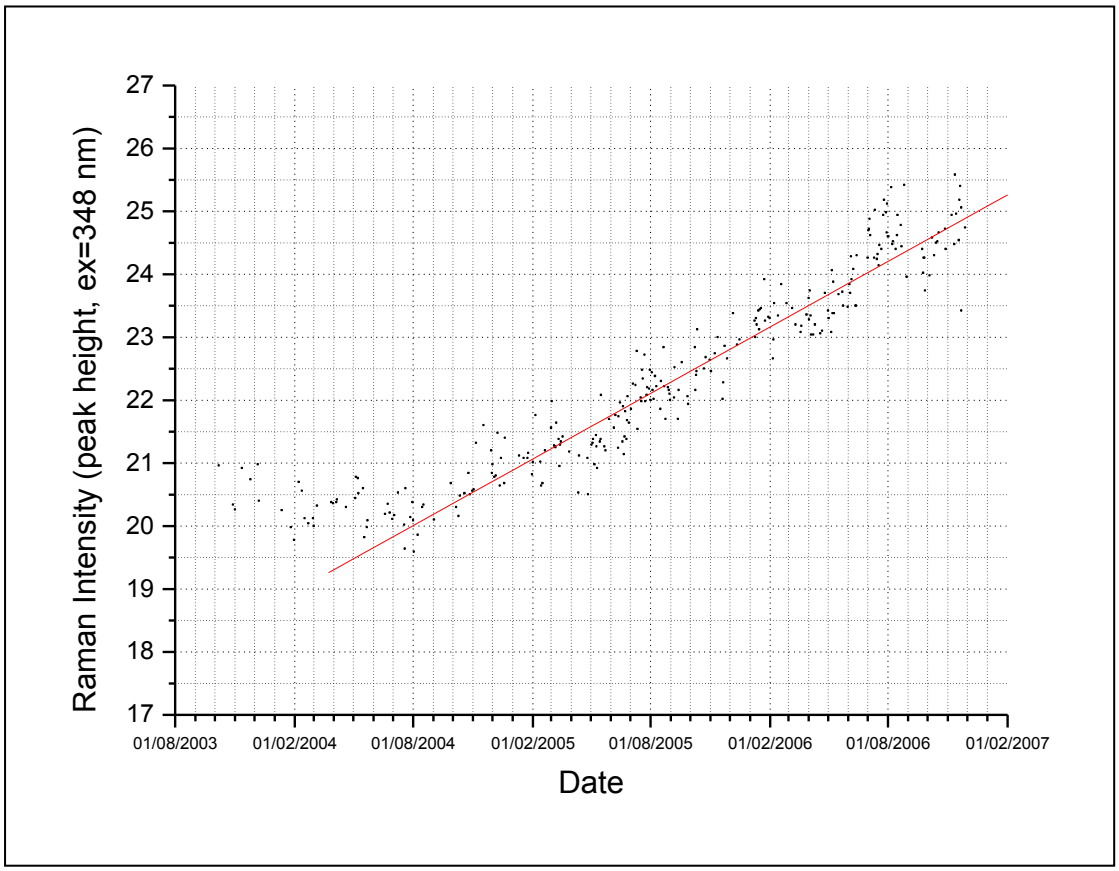


Figure 2.1: Plot of mean daily Raman value as measured on the Cary-Eclipse spectrophotometer by all laboratory users between August 2003 and February 2007 is observed (compiled by A. Baker), which encompasses the analytical period of this thesis. This plot shows an increase in Raman value over the analytical period indicating a drift in lamp output which is accounted for by normalisation of data to the Raman value on a daily basis

Analysis of 4ml of settled sample was undertaken in Studies 1, 2 and 3 using a 10mm path-length quartz cuvette. In Study 4, due to the small volumes of sample available a quartz micro-cuvette was used in which 400µl of sample was analysed at a path-length of 1cm. In Studies 1, 2 and 4 samples were not filtered prior to fluorescence to ensure that the full range of organic fractions was represented in the fluorescence data obtained. To minimise the effects of scattering by particles fluorescence was undertaken on settled samples. In

Study 3 samples were filtered prior to fluorescence as described in section 2.3.2.

The fluorescence character of laboratory "clean" water (distilled and/ or distilled deionised) was also assessed prior to each series of samples. In study 1 and 2 distilled water (condensed in a sterile system) was used and in studies 3 and 4 18MΩ deionised (condensed, filtered and UV treated) water was used. The distilled or 18MΩ deionised water was used for diluting excessively fluorescent samples and fluorescence intensities recorded so that an assessment could be made of the fluorescence contribution from the dilution water to the sample fluorescence intensities measured, and the contributory fluorescence intensity subtracted if necessary.

Data for four common fluorophores is presented in this work. The fluorophores studied are tryptophan-like/ protein-like and humic-like. Tryptophan-like fluorescence demonstrates two peak positions in the region of $\lambda_{ex/em}$ 280/350nm and $\lambda_{ex/em}$ 215-220/340nm which will be referred to as T_1 and T_2 respectively. Humic-like material is represented by two distinct fluorophores, commonly referred to in literature as humic-like and fulvic-like and which are referred to in this work as peaks C and A at peak regions $\lambda_{ex/em}$ 380/420-480nm and 260/380-460nm respectively (Coble, 1996). A further protein-like peak (peak B, tyrosine-like) is sometimes present in natural and polluted waters. Like the tryptophan-like peak it demonstrates a double peak character at peak positions $\lambda_{ex/em}$ 275/310nm and $\lambda_{ex/em}$ 220-225nm. However, these

peaks are highly influenced by the position of the Raman line and light source variability and it is not consistently present in samples analysed for this work. For this reason Peak B will not be discussed in this thesis. An example of river water EEM which illustrates common fluorescence peak positions is shown in Figure 2.2.

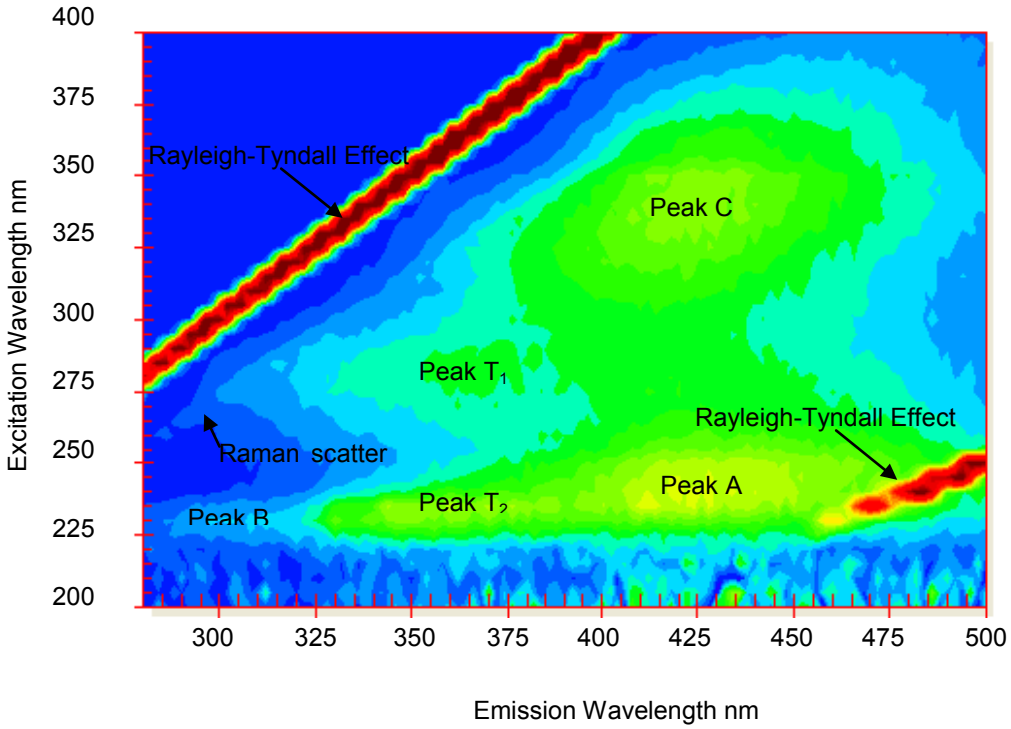


Figure 2.2: An illustration of the general peak positions of common fluorophores investigated in this work shown on a river water EEM. Peak B is subject to interference and is not investigated in this thesis. Peaks T_1 and T_2 indicate "protein-like" or "tryptophan-like" fluorescence centres, peaks C and A are commonly referred to as "humic-like".

In studies 1 and 2 samples were also analysed for fluorescence using an SMF-2 portable fluorimeter (Safe Training Systems, U.K). The SMF-2 uses a xenon flash light lamp. Excitation is targeted at 260nm through an interference filter with 85% transmission in the 260nm region and zero transmission at

350nm. A cut off filter is used to selectively advance the emitted light at wavelengths which have been identified as most appropriate for the application. The intensity of the fluorescence is measured by 250 detectors following a stationary grating. The SMF-2 is proposed as a field measurement device for identifying anthropogenic pollution (Baker et al., 2004).

2.1.2. Total Organic Carbon (TOC)

Undiluted samples were analysed for both total carbon and inorganic carbon and the total organic carbon then calculated by difference using a Shimadzu TOC-Vcpn analyser throughout. Total carbon was analysed by combustion of the sample at 680°C with a platinized alumina catalyst and the resulting CO_2 production measured. Total inorganic carbon was analysed by phosphoric acid digestion combined with CO_2 determination by IR detection.

From these analyses results the total organic carbon was calculated by total carbon – total inorganic carbon (TOC = TC - TIC). The instrument was calibrated prior to each analysis using a dilution series of total carbon and inorganic carbon 1 molar standards (Reagecon) and for each analysis the mean of 3 measurements was used.

2.1.3. Biochemical Oxygen Demand (BOD)

The BOD test is a measure of the oxygen depleting potential of an organic/ inorganic load in natural waters. It is a laboratory based biodegradation test which relies upon the presence of a viable microbial community which may be

naturally present in the sample or artificially introduced by addition of a seed, commonly a known volume of sewage effluent of known BOD. It is carried out under standard conditions (HMSO, 1988) and usually is run as 5-21 day test, 5 days being the most common period (BOD_5). The concentration of biodegradable material is calculated from the decrease in dissolved oxygen concentration over the 5 day period as labile organic material is oxidised by the microbial community. The BOD_5 test has a number of inherent difficulties associated with reliance upon the bacterial community and its sensitivity to environmental conditions. However the biggest inconvenience of the test is the 5 day test period which delays identification of potential pollution events.

BOD_5 was analysed in Studies 1, 2, 3 and 4 by the Environment Agency using the BOD_5 (ATU) method in which allylthiourea is added to inhibit nitrification within the sample, ensuring that the only inhibitor of organic material oxidation is dissolved oxygen concentration, as described in HMSO (1988). The minimum reported value (MRV) is quoted as $1.0 mgl^{-1}$ for this method (provided by email by A. Abed, NLS on 16/04/2010). However, it is not uncommon for standard dilutions to be undertaken in routine analyses, thus the MRV is not always reportable if the minimum dilution has been insufficient to achieve total oxidation of the bioavailable material. No indication is given of the level of accuracy of the BOD_5 method in either current Environment Agency method details (A. Abed by email 16/04/2010) or in the HMSO methodology (1988). However a rough indication may be obtained by assessing the standard deviation from the mean of analyses carried out on glucose/ glutamic acid standards quoted in HMSO (1988). This suggests that

the method is accurate to c. +/-8%. Dilution waters were seeded with a known quantity of treated sewage effluent of known $BOD_{5.}$ For all samples, seeding was conducted after a sub sample had been removed for fluorescence analysis.

2.2. Bottle Choice and Preparation

In all studies 50ml polypropylene bottles were used which had no cardboard or rubber inserts to minimise the risk of sample contamination with fluorescent material. In Studies 1 and 2 the bottles were washed in 10% HCl and distilled water and allowed to dry prior to use to remove any organic residues which might contaminate the sample. However the 18MΩ deionised water analysed in study 3 was stored in unwashed bottles to preserve the “sterile” status, as obtained from the manufacturer by avoiding possible microbial contamination during the washing and drying process. Also non-acid washed bottled were preferred for storing frozen samples in Study 4 as it was not known what effect on bottle life the acid washing method might have upon the bacterial population and the fluorescent organic material present.

2.3. Other Analytical Techniques

2.3.1. Study 2: Relationships between Fluorescence and Other Water Quality Parameters

Study 2 uses additional water quality data that was provided by the Environment Agency for samples from which sub-samples had been removed

for fluorescence analysis in Study1. The samples were collected by the Environment Agency as part of their regulatory activities and they undertake a range of different analyses depending upon the purpose of the sample. For that reason there is a wide range of diverse data, not all of which is available for every sample.

Water Chemistry

The Environment Agency undertakes analysis of a wide range of parameters in surface waters. In this work only the water chemistry parameters reported most often have been chosen for comparison with fluorescence to ensure the greatest range for statistical analysis. In the case of surface waters these parameters (in addition to BOD_5) are: Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), Total Carbon (TC), Total Inorganic Carbon (TC and TIC measured in the process of determining TOC at the University of Birmingham), Ammonia as N, suspended solids at 105°C, Alkalinity as $CaCO_3$, Nitrate as N, Nitrite as N, Orthophosphate as P.

The effluent water chemistry parameters which are most commonly reported (in addition to BOD_5) are: Total Organic Carbon (TOC), Total Carbon (TC), Total Inorganic Carbon (TC and TIC measured in the process of determining TOC at the University of Birmingham), Ammonia as N, suspended solids at 105°C.

Other analyses undertaken by the Environment Agency and at University of Birmingham are described in Table 2.1. Details of Environment Agency methods were provided by National Laboratory Services (NLS) by email on 11/04/2007. A number of the analyses by the NLS were undertaken spectrophotometrically using a “Konelab” discrete colourimetric analyser (Alkalinity, Ammonia, Chloride, Nitrite, Orthophosphate, Silicate and Total Oxidised Nitrogen information provided by A. Abed of NLS by email on 30/04/10). As this instrument clearly dictates a bias towards colourimetric methods these may not correspond with the methods commonly used in the water sciences community in which the most accurate method of analytical method is chosen on a parameter by parameter basis ion chromatography being the most common method. All colourimetric methods may be subject to interference from sample turbidity, precipitation of metal ions and absorbance of light in the analytical range by coloured samples.

Table 2.1: Details and methodologies for analyses undertaken by the Environment Agency on samples collected as part of the regulatory regime and used in Study 2 (Relationships between Fluorescence and Other Water Quality Parameters). Methodological details provided by the Environment Agency National Laboratory Services (NLS) by email on 11/04/2007.

Analytical Parameter	Method
Chemical Oxygen Demand (COD)	Samples are oxidised by refluxing with sulphuric acid and potassium dichromate with a silver salt to catalyse the oxidation of alcohol and low molecular weight acids. Mercuric sulphate is added and along with excess silver salt suppresses chloride interference and with it the effect due to ammonia. The mixture is refluxed and the residual dichromate (green colour of $Cr^{3+)}$ is determined photometrically.
Total Organic Carbon (TOC)	Analysed at University of Birmingham as described in section 3.1.2.
Total Carbon (TC)	Analysed at University of Birmingham as described in section 3.1.2.
Total Inorganic Carbon (TIC)	Analysed at University of Birmingham as described in section 3.1.2.
Ammonia as N	Ammonia reacts with salicylate and dichloroisocyanurate in the presence of sodium nitroprusside to form a blue colour, the intensity of which is proportional to the amount of ammonia present. Sodium citrate is added to mask possible interference from cations.
Suspended solids	Suspended matter is removed from a measured volume of sample by filtration under reduced pressure through a pre-washed, pre-weighed, pre-dried glass-fibre filter paper and determined gravimetrically after washing and drying at 105°C to constant weight.
Alkalinity as $CaCO_3$	The reagent uses methyl orange buffered with potassium hydrogen phthalate. Reduction in the red acid component of the indicator by carbonate/bicarbonates present in the sample is measured as a decrease in absorbance at 550nm
Nitrate as N	Nitrate is a calculated value determined by subtracting measured nitrite (NO_2-) from measured Total Oxidised Nitrogen.
Nitrite as N	Nitrite ions, when reacted with a reagent containing sulphanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride, in the presence of acid, produce a highly coloured azo dye that is measured photometrically at 540nm.
Orthophosphate as P	Orthophosphate reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex which, when reduced with ascorbic acid produces an intense blue colour, the absorbance of which is measured at 880nm.

2.3.2. Study 3: Changes in Fluorescence under Different Conditions of Light and Temperature

Dissolved Oxygen

Dissolved oxygen (DO) was measured using an YSI 55 membrane DO probe, which also measures temperature. In principle dissolved oxygen is measured through the change in current between 2 electrodes with application of a polarizing voltage. The current is proportional to the rate of oxygen diffusion through the membrane with rate of diffusion being influenced by the partial pressure of oxygen in the solution.

The instrument was calibrated before each use in fresh, well aerated mains tap water which was considered to be approximately at saturation point. Use of this as a calibration medium removed the uncertainty of potential bacterial growth in the calibration chamber, as the instrument was being used for various applications by a range of students, and allowed a level of confidence in calibration prior to field measurement.

Temperature

Temperature was also measured by the YSI 55 Dissolved Oxygen probe as the instrument automatically measures temperature of a sample to allow a temperature corrected dissolved oxygen value to be displayed.

pH

A WTW handheld multi-parameter instrument (Multi 350i) was used which can measure pH, temperature, conductivity and dissolved oxygen and which is suitable for field and laboratory use. The instrument was calibrated in pH 7 and 4.01 buffer solution each day prior to analysis.

Conductivity

Like pH measurement, conductivity was measured using a WTW handheld multi-parameter instrument. Calibration was performed each day prior to analysis using the calibration solution provided (1413µs/cm).

Filtration and bottling of samples for storage

Unfiltered sample was poured into sterile 50ml polypropylene bottles. The bottles were filled in such a way that they were completely full and minimal air was present as visible bubbles (both bottle and lid were filled, brought together and sealed rapidly).

Samples were filtered to obtain 3 fractions, unfiltered; nominally colloidal and dissolved (1.2µm); and dissolved only/ filter sterilised (0.1µm) using a vacuum filtration system. This is a rather simplistic allocation of fractions compared with that of Chow et al., (2005) who classify particulate organic carbon as 1.2-0.45nm, colloidal organic carbon as 0.45-0.1nm, fine colloidal organic carbon

as 0.1-0.025nm and dissolved organic carbon as <0.025nm, in the case of organic matter extracted directly from soils.

Whatman GF/C (nominal 1.2µm pore size) filters were chosen to remove particulate material while retaining the colloidal and dissolved material. Whatman Anopore 0.1µm membrane filters were used to filter sterilise samples (removal of all microbial fauna by filtration, hypothetically making samples immune to bacterial degradation). To isolate the nominally colloidal and dissolved fraction 1.2µm glass fibre filters were pre-washed with 50ml sample which was discarded prior to filtration of the main volume of sample. Half of the filtrate was then decanted into acid washed 50ml polypropylene bottles and sealed with minimal air inclusion as described above.

The vacuum filter units were rinsed thoroughly with 10% HCl and distilled water and the remaining 1.2µm filtrate was further vacuum filtered through Whatman Anopore membranes (nominal 0.1µm pore size) which were pre-washed with 50ml sample which was subsequently discarded. The remaining new filtrate was again decanted into acid washed 50ml polypropylene bottles as described above.

However Karlsson et al., (1998) suggest that the use of GF/C filters, even with filter pre-washing, causes the release of organic colloidal material to the sample filtered. Table 2.2 shows that these are not issues of concern in this work as there is no consistent increase in TOC or fluorescence observed through the filtration process, suggesting no release of fluorescent cellular

material or fluorescent material from the filters/ membranes. Inconsistencies in changes in fluorescence through the filtration process are most likely due to inadequate washing of the filter unit or contamination of the 10% HCl used for filter washing.

Table 2.2: Fluorescence Intensities and TOC values measured after sample filtration on Day 1of Study 3 (Changes in Fluorescence under Different Conditions of Light and Temperature) for different filtered fractions illustrating a lack of consistent contribution of organic or fluorescent organic material to filtered sample from filter papers.

Parameter	Fraction		River Dove	Repton Brook	River Tame	River Rea	Bourne Brook
			Fluorescence intensity (Arbitrary Fluorescence Units (AFU)				
T1	**Unfiltered**	**mean**	40.17	44.48	71.90	75.09	88.49
		s.d	1.32	2.22	3.48	4.90	9.97
	1.2µm	**mean**	39.01	51.19	82.29	75.62	92.31
		s.d	3.00	8.01	3.20	5.09	8.41
	0.1µm	**mean**	34.39	44.20	74.30	71.08	82.48
		s.d	2.79	6.30	2.40	2.82	16.58
T2	**Unfiltered**	**mean**	120.50	122.52	203.99	238.62	330.48
		s.d	10.22	6.55	6.46	8.57	17.67
	1.2µm	**mean**	103.45	128.67	206.65	253.31	260.01
		s.d	7.22	4.62	11.19	13.91	32.21
	0.1µm	**mean**	95.07	127.84	193.60	238.88	269.84
		s.d	17.52	4.85	2.44	8.93	40.29
C	**Unfiltered**	**mean**	92.47	79.44	95.87	96.46	172.61
		s.d	2.18	1.28	1.38	5.34	12.41
	1.2µm	**mean**	91.61	82.24	100.13	96.19	164.42
		s.d	1.00	3.03	3.23	2.12	0.95
	0.1µm	**mean**	83.51	74.69	99.60	99.40	163.87
		s.d	3.50	0.00	1.22	2.12	0.00
A	**Unfiltered**	**mean**	208.35	177.07	252.99	254.11	450.10
		s.d	7.57	4.67	10.85	6.36	16.74
	1.2µm	**mean**	201.13	177.63	250.06	261.06	435.35
		s.d	7.10	5.40	6.92	4.41	17.52
	0.1µm	**mean**	192.75	170.91	243.13	263.47	460.48
		s.d	7.37	10.92	3.02	5.15	61.03
TOC (mgl^{-1})	**Unfiltered**	**mean**	47.43	12.21	5.63	11.03	2.93
	1.2µm	**mean**	45.09	2.60	5.29	7.66	3.94
	0.1µm	**mean**	40.61	1.17	4.02	4.93	3.47

In existing literature it is most common for 0.2μm membranes to be used for filter sterilisation, however, it has been found that not all microbial fauna are retained by filters of this aperture (Hahn, 2004; Haller et al., 1999) and it is suggested that only pasteurisation can totally sterilise water samples (Little et al., 1987), however, it is highly likely that this would affect the character and structure of the organic matter present.

Microbiological Techniques

Basic materials are as outlined in the Environment Agency Guidelines, (2002). Although this is a drinking water application the basic principles were applicable to river waters which may contain a range of microbial fauna. However, incubation methodologies e.g. incubation temperature were substantially modified to make the microbial analysis relevant to the rest of the analyses undertaken.

The fundamental methodology for the microbiological element of this work is serial dilution of the sample and preparation of agar plates using a spread plate technique. Samples were filtered as described in section 3.3.2 and were incubated under the same conditions as water samples were stored. Colony forming units were counted after 24, 48, 72, 96, 264, 432 and 600 hours. Two types of media were used; nutrient rich (Yeast Extract Agar YEA) and nutrient poor (R2A), as proposed in the Environment Agency methodology for the analysis of the microbiology of drinking waters (Environment Agency, 2002).

For each media type 1 Litre of agar was mixed, in line with manufacturer's instructions, and autoclaved at 121˚C for 15 minutes. The media was allowed to cool to 50˚C (often using a water-bath set at 50˚C).

Around 20ml of cooled media was gently poured into triple vented sterile Petri dishes labelled with the date and media type, avoiding creation of bubbles. Petri dishes were left open to allow the media to dry for 15-20 minutes to reduce the amount of surface liquid in which colonies may migrate across the plate. Plate lids were replaced and the plates inverted to prevent condensation collecting on the media surface. Plates were stored in the refrigerator until required and were used within 1 week.

A serial dilution of each sample filtered fraction was undertaken using the technique as described in Cappuccino and Sherman (1996). Liquid transfer was undertaken using an auto micropipette with sterile disposable tips and serial dilution was undertaken in a sterile well-plate with dilution in this instance to 10^{-4}. The approximate dilution range required was determined by calculation of the dilution required to achieve 30-300 colonies per plate at a volume of 20µl per plate based on potential bacterial abundance in river waters being around10^{3} per ml river water (Coleman et al., 1974) depending upon water temperature, season and anthropogenic influences e.g. urbanisation, wastewater discharges.

Diluted sample was pipette onto an agar plate at room temperature and was spread using a sterile glass rod. The plate lid was replaced and the plate

inverted. Each filtered fraction for every sample was plated out 25 to 27 hours after sample collection and prior to plating up had been stored in the same light and temperature conditions as the water samples. The laboratory sterile distilled water used for sample dilution was also plated up as a control to examine the sterility of the dilution water.

Samples were stored in sealed Tupperware containers for incubation in the case of the samples incubated at 20˚C dark, 11˚C (light/dark) and 4˚C dark. The samples incubated at 11˚C dark were stored in plastic Petri dish sleeves in a sealed cardboard box (these were incubated in the same cabinet as the 11˚C light/dark samples and the sealed cardboard box excluded light during the light period of the cycle).

Basic counts of the number of colonies were carried out in line with fluorescence analysis 24, 48, 72, 96, 264, 432 and 600 hours after sample collection and a cumulative record kept. In the event that the number of colonies exceeded 300 per plate this was noted and no further count of that plate was undertaken. After each count the plates were returned to the individual incubation conditions. Total bacterial abundance in a sample was calculated from the dilution showing the greatest CFU count (not exceeding 300 CFU).

2.3.3. Study 4: Changes in Fluorescence with Freezing/ Thawing and Dehydration/ Rehydration

Environment Agency Contract Analysis

The Environment Agency laboratories at Starcross, Exeter were contracted to carry out a range of standard river water quality analysis on the samples collected for Study 3. This, in addition to TOC/ TC/ TIC measured in the original sample at the University of Birmingham on the day of collection and BOD_5 measured as previously described, provided a summary of the chemical water quality of each raw sample. The analyses undertaken by the Environment Agency are shown in Table 3.3 and method details were provided by NLS by email on 11/04/2007.

Table 2.3: Methodological notes for analyses undertaken by the Environment Agency for Study 4 (Changes in Fluorescence with Freezing/ Thawing and Dehydration/ Rehydration). Methodological details provided by National Laboratory Services (NLS) by email on 11/04/2007.

Method Name	Method protocol
Alkalinity $CaCO_3$	See section 3.3.1.
Ammonia as N	See Table 2.1.
Chloride	Chloride reacts with mercuric thiocyanate forming a mercuric chloride complex. Released thiocyanate reacts with iron (III) forming a red ferric thiocyanate complex. The intensity of colour produced, measured at 510nm, is proportional to the chloride concentration
Total Oxidised Nitrogen as N	The automated procedure for the determination of Total Nitrogen is based on the following reaction; the sample is mixed with an oxidising agent at 70°C and hereafter mixed with borax buffer and brought into an UV digester. On leaving, both organic and inorganic nitrogen compounds will have been converted to nitrate. The sample is then passed along copper cadmium wire to reduce nitrate to nitrite. The nitrite is determined by diazotising with sulphanilamide and coupling with a naphthylthylene diamine dihydrochloride to form a highly coloured azo dye. The absorption of this complex is measured at 540nm.
Nitrate as N	See Table 2.1.
Orthophosphate rv as P	See Table 2.1.
Silicate rv SiO2	Silicates in solution react with molybdate under acidic conditions to form a silicomolybdate complex. The complex is reduced by ascorbic acid to silicomolybdate blue. Interference by phosphate can be overcome by the addition of tartaric acid. The resultant compound is measured spectrophotometrically at 760nm. Molybdate reactive silicon includes mainly monomeric and dimeric silic acids and silicate.
Phosphate	Phosphorus in natural and waste waters will almost invariably be present in one or more of the following forms: Orthophosphate, Condensed phosphates e.g. pyro and poly phosphates, Organophosphates, Measured as orthophosphate as only this form will respond directly to spectrophotometric procedures.

Conductivity at 20 deg	The electrical conductivity of the sample is determined in a cell of known dimensions and will depend upon the concentration of ions. The unit of conductivity is siemen/metre although for convenience most measurements are reported in units of micro siemens (µs/cm). Conductivity is temperature dependent and therefore samples are quoted at 20□C.
pH	TThe pH of a solution is defined by the equation pH = -log a_H where a_H is the activity of hydrogen ions in the solution. As the hydrogen ion activities cannot be determined experimentally the pH of a solution is determined by measuring the electromotive force (emf) of a cell containing the test solution and comparing it with the emf of a similar cell in which the test solution is replaced by a standard buffer solution. Then: $\frac{pH(x) - pH(s)}{2.3026} = \frac{(Es - Ex)F}{RT}$ Where pH(s) is the pH of the standard buffer solution, pH(x) is the pH of the unknown solution, Ex is the emf of the cell containing the test solution, Es is the emf of the standard buffer solution, R is the gas constant, T is the absolute temperature, F is the Faraday constant
Turbidity	Light from a tungsten source, scattered by matter in the sample is measured at right angles to the incident beam. The intensity of the light scattered by the sample is compared with that measured for standard formazin suspensions and expressed as nephelometric turbidity units, NTU.
Nitrite as N	See Table 2.1.

2.4. Study Protocols

2.4.1. Study 1 Protocol – Relationship between Fluorescence and BOD_5

Figure 2.3 illustrates the analytical protocol for Study 1.

Sample site identification

The Environment Agency collects and analyses water samples as part of their regulatory routine. Sample batches consist of routinely sampled river waters, sewage effluents or trade effluents, or samples collected on a non-routine

basis in response to a potential pollution event. Approximately one litre of sample is collected and returned to the Environment Agency laboratory at the end of the day where they are registered and stored at 4˚C in a refrigerated room overnight prior to analysis. The following day various analyses are undertaken, including 5-day Biochemical Oxygen Demand (BOD_5) and a range of other chemical water quality parameters.

On 13 occasions over a period of 12 months (March 2005- February 2006), 50ml portions of registered and stored sample were decanted into 50ml polypropylene bottles (previously acid washed in 10%HCl, rinsed in distilled water and allowed to dry) prior to analysis and sent under cool, dark conditions (cool box) to the University of Birmingham for fluorescence analysis the following day. Sample collection, fluorescence analysis dates and types of sample analysed are listed in Table 2.4. Upon receipt of the samples at the University they were retained in the cool box and analysed for fluorescence and absorbance properties within 24 hours of receipt (which is within 48 hours of the start of BOD_5 analysis by the Environment Agency). Samples were refrigerated and stored for TOC analysis which took place 2 days later. Samples were disposed of after TOC analysis.

Table 2.4: Collection and fluorescence analysis dates of samples collected by the Environment Agency and analysed in Study 1 (Relationship between Fluorescence and BOD5). Also listed is the quantity of surface waters and pollution investigations/ effluents in each monthly sample batch.

Sample collection date	Fluorescence analysis date	Number of surface waters	Number of effluents
14 Mar 2005	16-17 Mar 2005	28	2
10-11 Apr 2005	14 Apr 2005	24	21
27-29 Apr 2005	04 May 2005	27	22
16 May 2005	18 May 2005	22	27
05-06 June 2005	08 June 2005	31	15
13 June 2005	15 June 2005	24	25
27 June 2005	29 June 2005	25	24
25-26 July 2005	29 July 2005	36	14
12 Sept 2005	14 Sept 2005	16	34
10 Oct 2005	12 Oct 2005	28	18
18-22 Nov 2005	23 Nov 2005	35	20
15-16 Jan 2006	18 Jan 2006	1	23
12-13 Feb 2006	15-16 Feb 2006	18	16

Sample preparation

In the majority of cases it was not necessary to undertake any sample preparation, although some samples required dilution to minimise the inner filtering effect and ensure that the fluorescence output measured was a direct measurement of the molecular emission, without energy dispersal and reemission by surrounding molecules. In this event the samples were diluted with the distilled water or 18MΩ deionised water that was used as the fluorescence blank. Fluorescence intensity results were corrected for the dilution factor. The fluorescence intensity of each fluorophore was then correlated with the BOD_5 value reported by the Environment Agency.

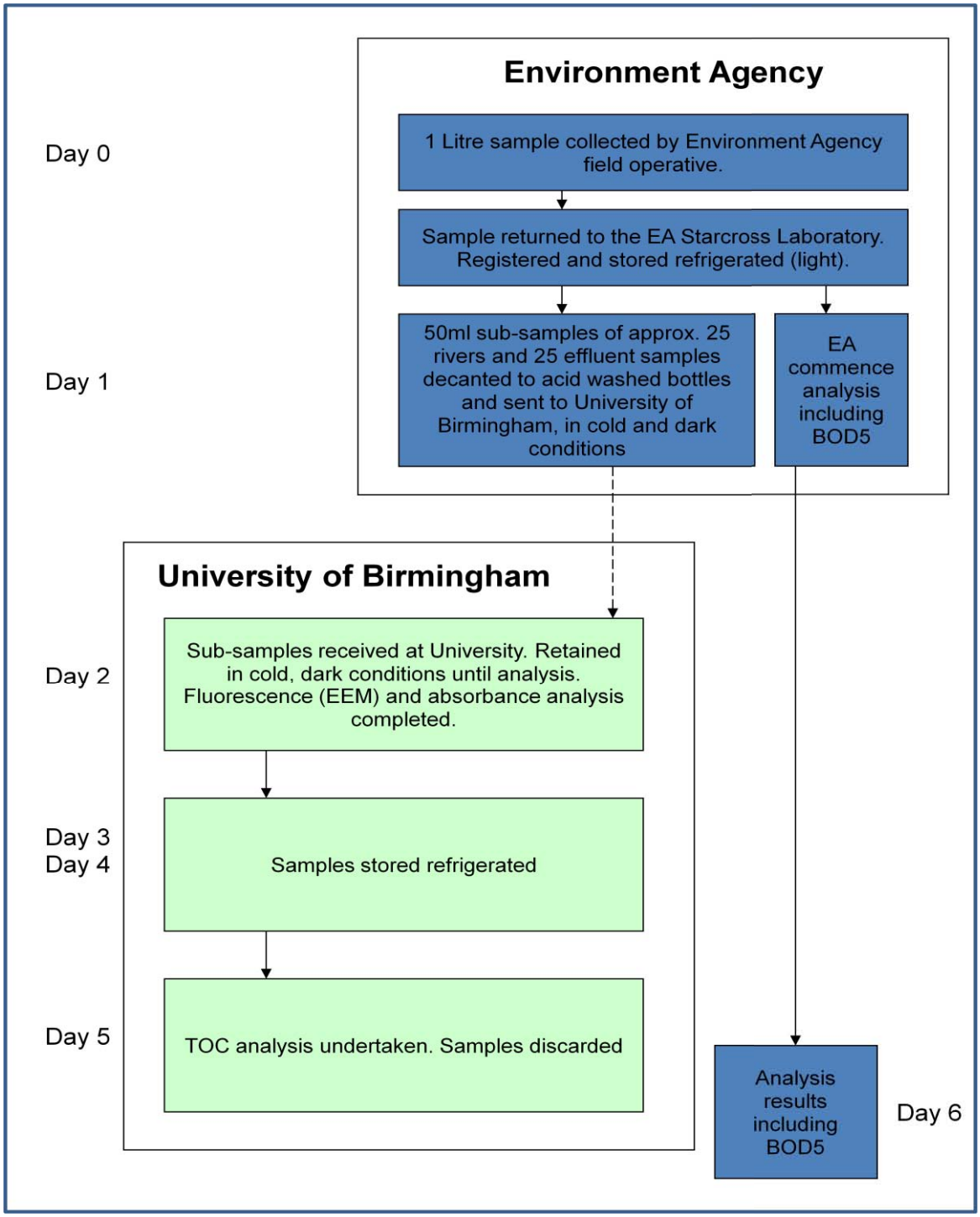


Figure 2.3: Pictorial representation of the analytical protocol for Studies 1 and 2, "Relationship between Fluorescence and BOD_5" and "Relationships between Fluorescence and other Water Quality Parameters".

2.4.2. Study 2 Protocol – Relationships between Fluorescence and other Water Quality Parameters

The study protocol is the same as study 1.

Sample site identification

All samples investigated in Study 1 regardless of location or time from collection.

Sample preparation

As described in section 2.4.1.

2.4.3. Study 3 Protocol – Changes in Fluorescence under Different Conditions of Light and Temperature

Figure 2.5 illustrates the analytical protocol for Study 3.

Sample site identification

Samples were collected from 5 rivers in the Midlands during summer low flow conditions (between May 2006-September 2006), and 1 control sample of 18MΩ deionised water (November 2006) for analysis of changes in fluorescence over time. The rivers were chosen to include a range of urban and rural characteristics, distances from source and chemical water quality grades. The upstream and downstream grid reference of each river reach, the 2003-2005 average Environment Agency chemical water quality results and, where available, Water Framework Directive risk assessments are shown in Appendix 1.

Sample collection and storage

25 Litres of water sample were collected using a bucket (previously rinsed with sample) and decanted into an HDPE carboy, previously rinsed with tap water and sample. In the majority of cases it was possible to access the river and so samples were collected from approximately 30cm depth, approximately 1.5m from the bank, in flowing water. Where it was not possible to enter the river sample was collected by suspending the bucket from a bridge over the river at approximately the same distance from the bank.

The sample was analysed for dissolved oxygen, temperature, pH and conductivity in the field. Samples were then returned directly to the laboratory, at ambient temperature. Filtration and fluorescence analysis was carried out a maximum of 5 hours after sample collection, and samples were put to incubate under each of the storage conditions approximately 8 hours after sample collection.

Sample preparation

Upon return to the laboratory the carboy was shaken to agitate the sample and one litre was decanted into a plastic bottle provided by the Environment Agency. The sample was packed in ice packs in a cool box and the box mailed to the Environment Agency for BOD_5 analysis the following day.

The remaining sample was filtered and prepared for storage and fluorescence analysis as described in section 2.2.1. The bottled, filtered fractions were placed to store under specific conditions which were chosen to replicate natural and experimental conditions. The storage conditions were:

20°C Dark – replicates the conditions of the BOD test under which bacterial activity and organic matter biodegradation is measured.

11°C Light/ Dark cycle – represents the environmental average temperature in the region (9°C -11°C depending upon elevation) (The Met Office) and diurnal cycles. Daylight hours were 7am to 10pm as these were the conditions

at the time of collection of the first sample. These conditions were created in an environmental cabinet in which 8 OSRAM lamps (Lumilux fluorescent daylight tube ref. 950), which emit in the visible/ white light range violet to red, were installed. The output spectrum of the lamps (L. Kingdon email communication 27/04/2006) can be seen in Figure 2.4 as can the absorbance spectrum of the bottles which demonstrates that at the output spectrum of the lamps the absorbance of light by the bottles is minimal, exposing the sample to the majority of wavelengths of light. The bottle absorbance profile was provided by R. Sturman of the Department of Chemistry at the University of Birmingham, by email on 22/02/2007.

11°C Dark – as a dark control to determine the influence of light on the samples stored under the previous conditions.

4°C Dark – replicated the most common storage conditions for water samples prior to fluorescence analysis.

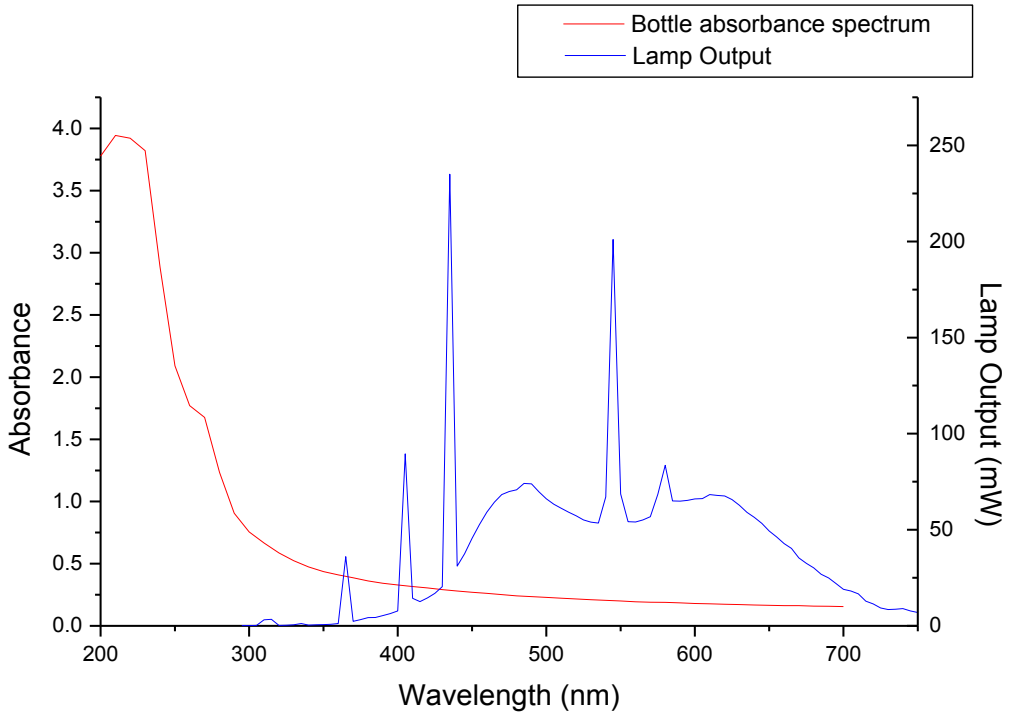


Figure 2.4: The output spectrum of the Osram lamps (L. Kingdon email communication 27/04/2006) plotted against the absorbance spectrum of the bottles used for sample storage, demonstrating that at the output spectrum of the lamps the absorbance of light by the bottles is minimal, exposing the sample to the majority of wavelengths. The bottle absorbance profile was provided by R. Sturman of the Department of Chemistry at the University of Birmingham, by email on 22/02/2007.

The temperature in each of the artificial environments was continuously monitored using TinyTag continuous temperature loggers (Gemini Data loggers), and was logged at 5 minute intervals for the 600 hours of the experiment. The deviation from the preferred temperatures is illustrated in Table 2.5.

Table 2.5: Mean temperatures recorded using Gemini TinyTag loggers under each of the defined storage temperatures (4°C, 11°C and 20°C) in Study 3 “Changes in Fluorescence under Different Conditions of Light and Temperature” and the standard deviation from the mean.

		River Dove	Repton Brook	River Tame	River Rea	Bourne Brook
4°C	mean °C	4.60	4.36	4.58	4.16	4.48
	S.D	0.8637	0.8437	1.1141	0.7584	0.6920
11°C	mean °C	12.86	13.32	13.06	12.68	12.63
	S.D	0.7455	0.9666	0.8832	0.8225	0.6851
20°C	mean °C	18.81	19.31	18.77	18.79	18.45
	S.D	0.3680	0.9401	0.5003	1.5246	0.2869

Sample Analysis

50ml of each filtered fraction was retained for fluorescence and TOC analysis on day 1 as described in sections 2.1.1 and 2.1.2. An additional 50ml was retained (sealed with minimal air) and refrigerated overnight and then plated out for microbiological analysis as described in section 2.3.2.

After 24, 48, 72, 96, 264, 432 and 600 hours from sample collection a 50ml sample of each filtered fraction from each storage condition was analysed for fluorescence, pH, DO, temperature, conductivity and TOC using methods previously described. The sample was then discarded.

CFU counts were undertaken on every plated sample from every storage condition 24, 48, 72, 96, 264, 432 and 600 hours after sample collection as described in section 2.3.2. The plates were then returned to storage for analysis on subsequent days.

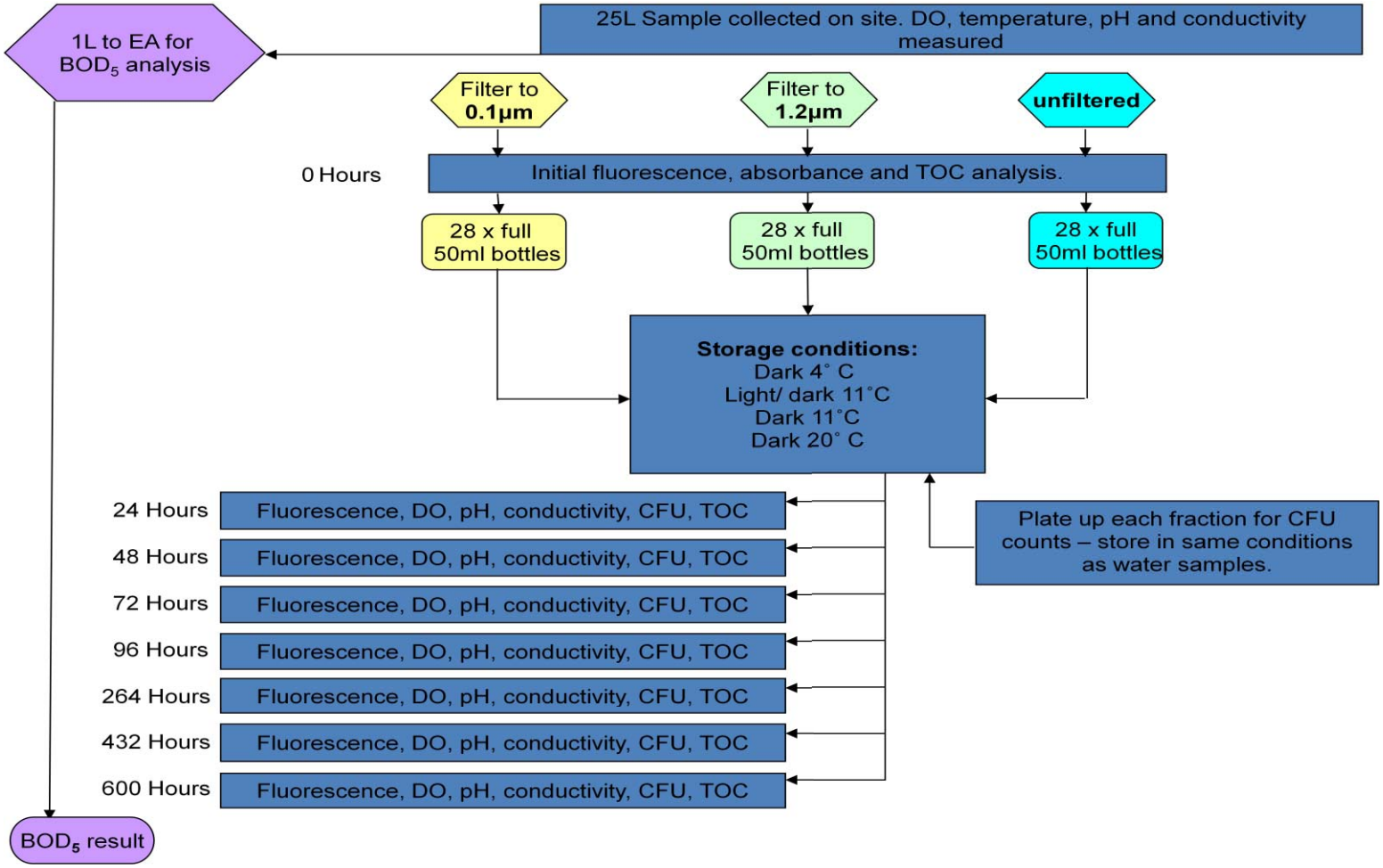


Figure 2.5: Analytical protocol for Study 3 “Changes in Fluorescence under Different Conditions of Light and Temperature.”

2.4.4. Study 4 Protocol – Changes in Fluorescence with Freezing/ Thawing and Dehydration/ Rehydration

Figure 2.6: illustrates the analytical protocol for Study 4.

Sample site identification

Water samples were collected from 13 surface waters in the Midlands between November 2006 and February 2007 for analysis of changes in fluorescence with dehydration/ rehydration and freezing/ thawing. The rivers were chosen to include a range of urban and rural characteristics and, as it was not possible to gain any sort of temporal replicate in the period of time available, it was decided to work with a larger spatial distribution of samples. The watercourses sampled can be grouped into geographically similar areas, and these groups ultimately feed into the same higher order watercourses. The grid reference and date of collection of each sample collected for this analysis is shown in Table 2.6.

Table 2.6: The common names, grid references and sampling dates of surface waters sampled for analysis in Study 4 “Changes in Fluorescence with Freezing/ Thawing and Dehydration/ Rehydration.”

Sample Name	Sample type	Sample Location GR	Date sampled
River Tame	Surface Water	SP 174 914	20 Nov 2006
Wood Brook	Surface Water	SK 033 815	11 Jan 2007
River Rea	Surface Water	SP 067 840	20 Nov 2006
Harbourne Brook (N)	Surface Water	SP 026 836	20 Nov 2006
Harbourne Brook (S)	Surface Water	SP 026 836	20 Nov 2006
Vale Lake	Surface Water	SP 052 847	20 Nov 2006
Bartley Brook	Surface Water	SK 022 829	11 Jan 2007
Merritt’s Brook	Surface Water	SK 034 811	11 Jan 2007
River Trent	Surface Water	SK 254 221	11 Jan 2007
Repton Brook	Surface Water	SK 307 265	20 Nov 2006
Hilton Brook	Surface Water	SK 241 306	11 Jan 2007
River Dove	Surface Water	SK 214 295	20 Nov 2006
Alder Brook	Surface Water	SK 236 276	11 Jan 2007
18MΩ deionised water	Control		20 Nov 2006

Sample Collection and Storage

Two litres of each water sample were collected directly into two unused, unwashed 1litre bottles. As no sampling aids were used the distance of sampling from the bank was <1m and sampling depth was around 10-20cm. No field analysis of samples was undertaken other than a visual assessment of low or high flow status and visual or odour indicators of obvious pollution incidents.

Samples were returned to the laboratory and stored in the refrigerator until analysis. One full 1 litre bottle of each sample was sent to the Environment Agency for analysis in cold, dark conditions (cool box) within 6 hours of sample collection. These samples were registered within 2 days and full

chemical water quality analysis as described in section 2.3.3 was undertaken. The reported parameters and (abbreviated titles) were Alkalinity as $CaCo_3$ (Alk); Ammonia as N (AmN); BOD_5; Chloride (Chl); Total Oxidised Nitrogen (TON); Nitrite as N (NO_2^-); Orthophosphate as P (Orthop); Silicate as SiO_2 (SiO_2); Phosphate (Phosp); Conductivity (Cond); pH; Turbidity (Turb); Nitrate as N (NO_3^-).

Sample Preparation

In order to be more representative of water in the natural system samples were not filtered prior to analysis. Within 6 hours of sample collection 40ml of unfiltered sample was decanted into new unwashed, sterile 50ml polypropylene bottles in duplicate for each sample which allowed a margin for volume increase during freezing. The bottles were then placed in a laboratory freezer at approximately -20°C (batch 1, 20/11/2006 – 09/01/2007 = mean temperature -19.9°C +/- 0.67°C, batch 2, 11/01/2007 – 01/02/2007 = mean temperature -19.71°C +/- 0.52°C).

8ml of unfiltered sample was also decanted into sterile Petri dishes in duplicate and placed, uncovered, in an oven which had been previously sterilized by washing with 70% ethanol/ MIS. The over temperature was maintained at around 30°C (batch 1, 20/11/2006 – 09/01/2007 = mean temperature 36.58°C +/- 0.46°C, batch 2, 11/01/2007 – 01/02/2007 = mean temperature 31.57°C +/- 1.06°C) An ambient temperature of 30°C was chosen as it was considered that this was sufficiently environmentally relevant

to produce meaningful results while also dehydrating the sample within a time scale that allowed for the experimental phase to be completed within the time available. For the same reason 8ml of sample was decanted for dehydration and rehydration analysis as this was found to dehydrate overnight under the temperature conditions. This allowed a rapid recovery of results.

In addition, 40ml of sample was decanted into unwashed, sterile 50ml polypropylene bottles and stored under refrigerated conditions as a control, against which to measure changes in fluorescence as a result of freezing and thawing and dehydration and rehydration. These samples were refrigerated at around 4°C, in the dark, and were analysed again for fluorescence only on the last day of the test.

Sample Analysis

24 hours prior to analysis the frozen samples were removed from the freezer and allowed to thaw in an environmental cabinet in cycles of light and dark at approximately 11°C. One hour before analysis commenced the dehydrated samples were covered with lids, collected with the thawed samples and taken to the fluorescence laboratory. They were stored at room temperature under laboratory lights until analysis. The dehydrated samples were rehydrated approximately 1 hour prior to analysis by the addition of 8ml of 18MΩ deionised water for which fluorescence EEMs had previously been determined and the dishes were covered until analysis. No preparation was undertaken prior to fluorescence analysis for the frozen samples.

It was necessary to dilute some samples due to their turbidity or very high fluorescence intensities. In the case of both frozen and dehydrated samples the test was then destructive, with a volume of sample being removed from the bulk and not returned. This is considered to be of negligible impact as for freezing/ thawing each fluorescence analysis used only 400µl, from a total volume of 40ml (1%) and for dehydration 400µl of 8ml (5%).

Following fluorescence analysis the thawed samples were returned to the freezer and the rehydrated samples were returned, uncovered to the oven at 30°C to undertake another cycle of freezing and dehydration respectively. This cycle was repeated five times with fluorescence analysis being undertaken after each cycle. Dates of analysis are shown in Table 2.7.

Table 2.7: Dates upon which fluorescence analysis of frozen/ thawed and dehydrated/ rehydrated samples was undertaken

Samples collected 20/11/06		**Samples collected 11/01/07**	
Dehydrated/ rehydrated	Frozen	Dehydrated/ rehydrated	Frozen
21 Nov 2006	22 Nov 2006	16 Jan 2007	16 Jan 2007
22 Nov 2006	24 Nov 2006	23 Jan 2007	23 Jan 2007
24 Nov 2006	01 Dec 2006	25 Jan 2007	25 Jan 2007
01 Dec 2006	08 Dec 2006	31 Jan 2007	31 Jan 2007
08 Dec 2006	09 Jan 2007	01 Feb 2007	01 Feb 2007

Control samples which had been stored under refrigerated conditions in the dark from the day of collection for the duration of the freezing/ thawing or dehydration/ rehydration analysis were also analysed for fluorescence properties during cycle 5 analysis.

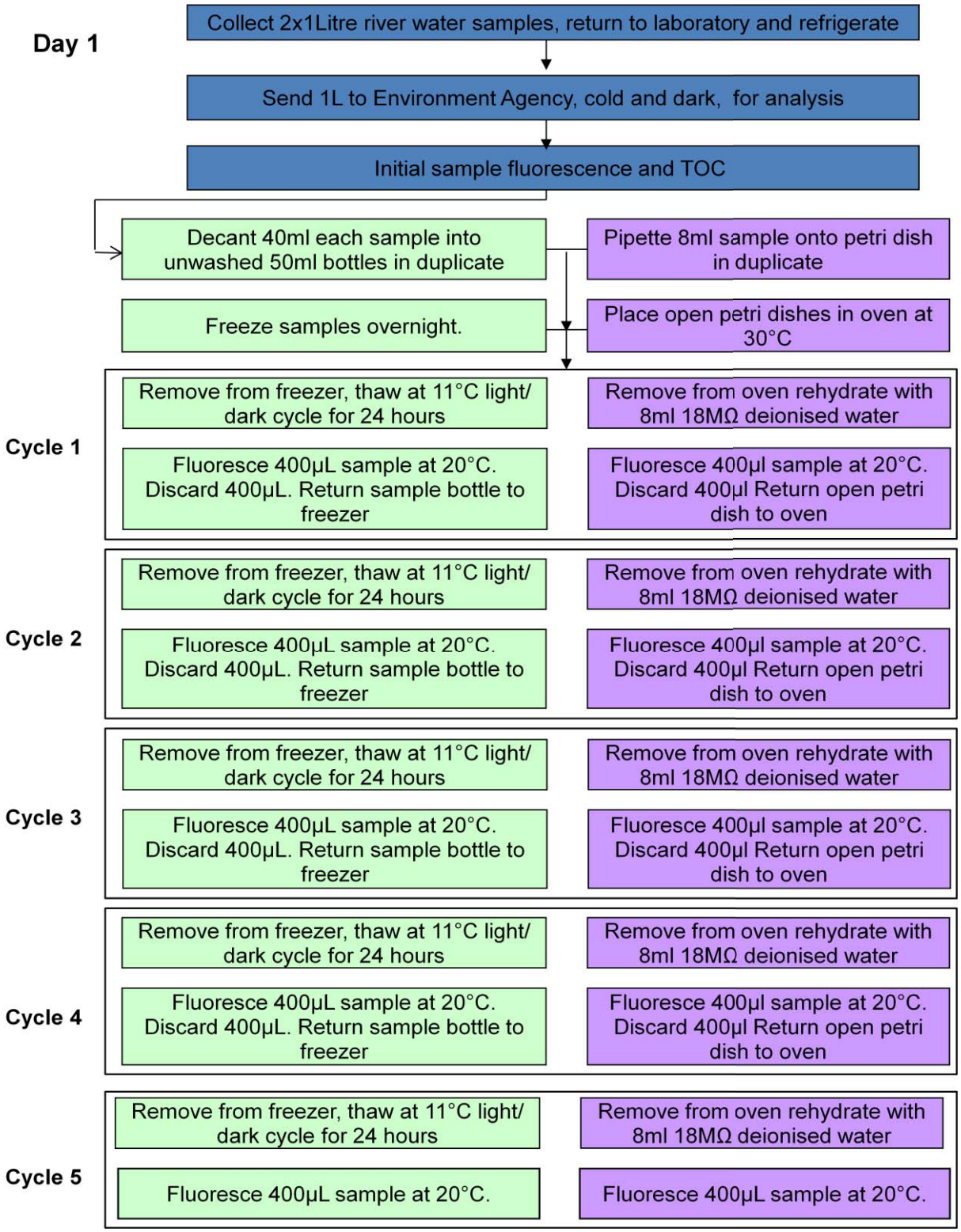


Figure 2.6: Pictorial representation of the analytical protocol for Study 4 “Changes in Fluorescence with Freezing/ Thawing and Dehydration/ Rehydration.”

3. RELATIONSHIP BETWEEN FLUORESCENCE AND BOD_5

Rationale

An investigation into the relationship between organic matter fluorescence and BOD_5 in fresh waters and effluents, undertaken with a view to informing the existing body of research into the potential of fluorescence spectroscopy as a laboratory or field surrogate for the BOD_5 test.

3.1. Introduction

This chapter will present results pertaining to relationships between the fluorescence profile of surface waters and sewage effluents and their biodegradation potential as measured by the five day Biochemical Oxygen Demand test (BOD_5). Strong arguments have been developed over time by a number of authors from various different fields which suggest that it is possible to use fluorescence as an indicator of BOD_5 (See Table 3.1). This chapter aims to further inform this debate by presenting correlations between fluorescence parameters and BOD_5 for a sample set which is larger, more geographically diverse and includes greater range of sample types than any previous work.

3.2. Literature Review

3.2.1. BOD_5 and Fluorescence Analysis

As previously described in section 2.1.3 BOD_5 is a measure of the activity of the microbial community present in water, supported by a labile organic substrate. The amount of biodegradable material present is reflected by the volume of oxygen used by microbial respiration over a pre-determined period of time in a sealed system in which nutrient levels are augmented so that oxygen availability is the only limiting factor. The use of fluorescence as a water quality indicator was initially proposed by Comber et al., (1996) who found no meaningful correlation. This is now thought to be due to the choice of an inappropriate fluorescence excitation/ emission pair.

Fluorescence spectroscopy has also been used for a number of years in the study and identification of microbial communities, either fingerprinting and characterising particular species (Seaver et al., 1998; Smith et al., 2004; Elliott et al., 2006), species identification (Gray et al., 1998; Leblanc et al., 2002) or in process monitoring (Farabegoli et al., 2003; Saadi et al., 2006). Cammack et al., (2004) and Elliott et al., (2006) also showed that tryptophan-like fluorescence is directly related to microbial activity and may represent either a product of or substrate for the microbial community. Reynolds and Ahmad, (1997) determined that the sewage treatment process reduced peak T intensity to a much greater extent than the humic-like A and C peaks. This suggests that the T peak in untreated sewage, derived from anthropogenic activity, represents fresher, less degraded material with a high potential for

oxidation. As the BOD_5 test is a microbial based assessment of polluting load, there should be a correlation between “microbial” tryptophan-like fluorescence and absolute BOD_5 measurement and therefore fluorescence of the tryptophan-like peak should be an indicator of the BOD_5 (or organic load) of a water.

Further work investigating the relationships between fluorescence and BOD_5 has shown that correlations between the tryptophan-like peak and BOD_5 do exist for a wide range of sample types (Ahmad and Reynolds, 1999; Reynolds, 2002; Baker and Curry, 2004; Baker and Inverarity, 2004; Hur et al., 2008) in a range of water types. Waste waters including sewage effluents (Reynolds and Ahmad, 1997; Reynolds, 2002; Chen et al., 2003), farm wastes (Baker, 2002b) and landfill leachates (Baker and Curry, 2004; Lu et al., 2009) have been found to be rich in microbial derived T (and B) fluorescence and these peaks have been used as tracers of waste waters in natural waters (Baker and Inverarity, 2004; Baker et al., 2004; Lapworth et al., 2008) and in potable water (Henderson et al., 2009).

Where relationships have been found to exist they have been significant. However, the majority of previous work has investigated the relationship in “stronger” waters e.g. wastewaters and sewage effluents often collected from the same sites. These works show that on a site by site basis good relationships exist, however most do not address the influence of variable site character upon the use of fluorescence as an indicator of BOD_5. Within a single site a correlation coefficient of 0.97 was considered to be “less than

desirable” by Ahmad and Reynolds, 1999 and the variance within the dataset was attributed to environmental factors. This chapter seeks to investigate the strength of the correlation between fluorescence and BOD_5 for a set of data in which diversity in water type, sites character, seasonal difference in flow rate and input and a range of other influential factors is high. It is the largest study to date which analyses subsets of the same collected sample, from the largest geographical area with the largest seasonal variation.

Previous works have identified different specific wavelengths of excitation and emission in the study of fluorophore T_1 and its relationship with BOD_5. The variation in wavelengths is likely to be due to the physical characteristics of individual samples such as pH, metal ions, sample concentration (Vodacek and Philpot, 1987). These factors have not been analysed on a sample by sample basis for this (or any previous) work. For this reason the wavelengths identified in each individual body of work are presented with no correction for the possible contributory factors. Table 3.1 illustrates the sample types and number of samples used in studies of the BOD_5/ tryptophan-like fluorescence relationship.

Table 3.1: A summary of published work relating fluorescence to BOD_5 illustrating the correlations coefficients determined in the work, sample types and number of samples used in studies of the BOD_5/ tryptophan-like fluorescence relationship.

Fluorescence Intensity at ex/ em	Correlation Coefficient (r)	Number of Samples	Comments	Water Type	Author
Ex 250nm, 350nm Em 430nm	-	c.200 samples	No relationship. Wrong wavelength pairs examined.	River, sewage and industrial effluent.	(Comber et al., 1996)
280/340nm (T_1)	0.94 - 0.97	129 samples, 3 sites	Relationship is site specific.	Composites of untreated, settled and treated waste from 3 sewage works.	(Reynolds and Ahmad,1997)
248/340nm (T_2)	0.97	25 samples, 1 site	Site specific relationship expected.	Single sewage works.	(Ahmad and Reynolds, 1999)
280/350nm (T_1)	0.96 - 0.99	101 samples, 2 sources		Single sewage works and synthetic sewage.	(Reynolds, 2002)
220-230nm/ 340-370nm (T_2)	0.94 - 0.98	40 samples, 3 sites	Relationship site specific.	3 Landfill leachates.	(Baker and Curry, 2004)
220/350nm (T_2)	0.85	434 samples, 62 sites	Samples not paired.	River waters	(Baker and Inverarity, 2004)
“Protein-like”	0.892 and 0.901	54 samples,18 sites		Headwater sample, urban river, effluent, reservoir discharge	(Hur et al., 2008)
280/350nm (T_1)	0.906	294 samples, 267 sites	Paired samples.	141 effluents, 124 surface waters, 2 pollution incidents.	(Hudson et al., 2008)

3.3. Relationships between Fluorescence and BOD_5

3.3.1. Description of Samples

574 samples were analysed for fluorescence and BOD_5 in total. The majority of samples were collected in South West England. Some samples were, however, collected outside this region and are excluded from consideration as the influence of geography and proximal relationships on sample character is important in this work. Fluorescence and BOD_5 data (with other water quality parameters used in Chapter 4) for all samples are shown in Appendix 2.

Other samples are also excluded from these results. Samples with oxygen depletion less than $1mgl^{-1}$ (the Environment Agency MRV) over 5 days are quoted by the Environment Agency as a BOD_5 value of "<1" and have no true numerical value with which to correlate fluorescence intensity. Furthermore, samples for which inappropriate dilution factors have been used, leading to incomplete oxidation of organic material may return a "less than" figure, with no numerical value. In essence such samples exhibit a zero/ near zero or unrecorded biochemical oxygen demand as determined by the standard five day BOD_5 test. For clarity, correlation between fluorescence intensity and BOD_5 data has been performed only on samples exhibiting a numerical BOD_5 value. By also excluding the samples returning no numerical value the total number of suitable samples is reduced to 294 (135 surface waters and pollution investigations and 159 effluents) from 267 sites. All correlations presented in this work are for this set of 294 relevant samples.

However, missing BOD_5 may be remedied in a number of ways and the implication of these methods is investigated. A number of correlations were carried out for the full set of 574 samples. Three approaches were taken to allocating a BOD value where a “less than” value had been returned: BOD = 0, BOD = a randomly generated value between 0 and the value that BOD was “less than” e.g. between 0 and 1; BOD = 0.5 MRV.

Correlations between BOD_5 variations and fluorescence intensities for peaks T_1, T_2, C and A are show in Table 3.2.

Table 3.2: Correlation coefficients (Spearman's Rho, r) between fluorescence intensity of peaks T_1, T_2, C, A and BOD_5 for all 574 analysed samples when missing BOD values are allocated as BOD =0; BOD = randomly generated value between 0 and the value that BOD was "less than" e.g. between 0 and "<"1; BOD = 0.5MRV. (R^2 values are shown in parentheses). Correlations significant at the 0.01 level (2-tailed) are highlighted. P values indicate the 2-tailed probability of the difference between the BOD as reported vs. fluorescence correlation being significantly similar (at the 95% level) to the manufactured BOD/ fluorescence correlation.

Spearman's Rho (r)	N	**Peak T_1**	**Peak T_2**	**Peak C**	**Peak A**	**SMF-2 T_1 Region**
BOD as reported	325	.83 (.69)	.84 (.71)	.74 (.55)	.67 (.45)	.81 (.66)
BOD=0	574	.70 (.49)	.63 (.40)	.56 (.31)	.52 (.27)	.64 (.41)
BOD=random	574	.80 (.64)	.75 (.56)	.67 (.45)	.63 (.40)	.76 (.58)
BOD=0.5MRV	574	.70 (.49)	.63 (.40)	.56 (.31)	.52 (.27)	.64 (.41)
z-score (BOD as reported vs. BOD random)		1.28	3.56	2.01	0.99	1.88
P (2-tailed)		.20	0.00	0.04	.22	.06

The three different approaches to allocating BOD_5 values demonstrate that random allocation of a BOD value to replace missing data gives correlations with the BOD as reported data which are significantly similar at the 95% level for peaks T_1, peak A and the SMF-2 T_1 region, at the 99% level for peak C and with no statistical similarity for peak T_2. The mean, standard deviation and standard error for the various BOD datasets are presented in Table 3.3. The mean BOD value is lower in manufactured data, although the standard deviation of the data is still quite high, probably because these approaches make more surface water sample data available for analysis. As the agreement between actual and "manufactured" BOD data is not consistent for all peaks I will use only data which returned a numerical BOD_5 value in this

chapter. In addition, for the preliminary sections of this chapter geographical relativity is important, so only samples from the South West of England (nominally identified by Ordnance Survey Grid references SS, ST, SW, SX and SY) will be used. Furthermore, samples collected in July 2005 were excluded as excessive time elapsed between sample collection and analysis (in excess of 800 hours).

Table 3.3: Mean, standard deviation and standard error values for the full 574 samples of each of the approaches to BOD_5 reporting (missing BOD values replaced with BOD=0, BOD=randomly assigned value as previously described, BOD = 0.5MRV) demonstrating that manufactured BOD data changes the distribution of the dataset probably as a result of making a greater number of surface water sample data available for analysis.

	BOD as reported	**BOD = 0**	**BOD random**	**BOD = 0.5MRV**
N	325	574	574	574
Mean	9.14	5.76	6.15	5.95
Standard deviation	28.31	22.90	22.84	22.86
Standard error	1.57	0.96	0.95	0.95

Correlation coefficients differ to those cited in the published BOD_5/ fluorescence paper (Hudson et al., 2008) as one BOD_5 value used in the paper was found to have been incorrectly transposed (sample 761179 BOD_5 value in paper = 4.65, corrected to 1.65 for thesis).

As described in section 2.1.1.some samples were diluted in order to obtain a recordable fluorescence intensity value for all peaks. Data and correlations presented are for samples at the lowest dilution at which all peaks were visible and demonstrated measurable fluorescence values using the Cary Eclipse.

Measured fluorescence intensity is multiplied by the Raman correction value (see section 2.1.1) and the dilution factor and the resulting calculated fluorescence intensity correlated with BOD_5.

3.3.2. General Fluorescence Characteristics

Fluorescence EEM analysis of the surface waters collected showed that they generally contained humic-like material with peak A ($\lambda_{ex/em}$ 260/380-460nm) being more intense than peak C ($\lambda_{ex/em}$ 380/420-480nm). Tryptophan-like peaks T_1 and T_2 were often present and varied in fluorescence intensities. T_2 fluorescence intensities were always greater than T_1 (average ratio 2.80). Sewage effluents contained peaks T_1 and T_2 at greater intensities than surface waters with T_2 intensity exceeding that of T_1 (average ratio 1.77). In some samples peak A was obscured by the fluorescence of peak T_2 only becoming measurable as a peak after dilution. Throughout this study only 2 surface water samples and 15 effluent samples required dilution prior to fluorescence analysis.

3.3.3. Relationship between Fluorescence Intensity as Measured on Cary Eclipse Spectrophotometer and BOD_5

Correlation

Table 3.2 illustrates the relationships between BOD_5 and fluorescence intensities for all samples and surface water and effluent subsets among the 294 samples. Note all correlation coefficients "r" are non-parametric (Spearman's Rho) as the BOD_5, and fluorescence data do not conform to a

normal distribution as defined by visual assessment of a distribution histogram and the Kolmogorov-Smirnov test (the fluorescence intensity value (T_1) D(294) = 0.21, p <.0001, and BOD_5 as reported D(294) = 0.40, p <.0001, are both significantly non normal) and so a ranked correlation is a more appropriate method of statistical analysis.

Table 3.4: Correlations between BOD_5 and fluorescence intensity, for 4 common fluorophores measured on Cary Eclipse spectrophotometer in 294 samples. R^2 values in parentheses. Significant correlations at the 0.01 (2-tailed) level are highlighted.

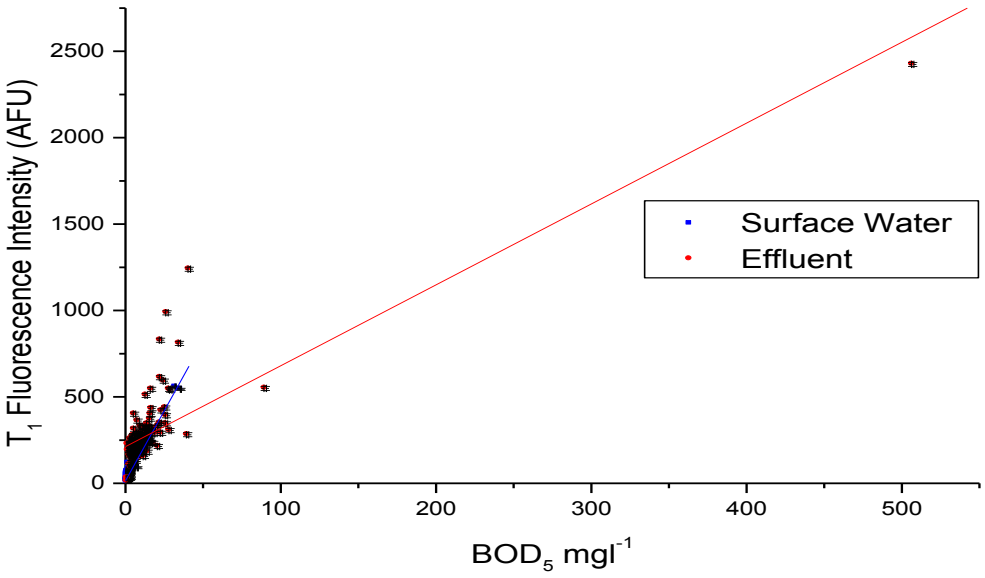
Spearman's Rho (r)	**Number of samples**	**Peak T_1**	**Peak T_2**	**Peak C**	**Peak A**
All Samples	294	.90 (.82)	.84 (.71)	.77 (.59)	.72 (.51)
Surface water	135	.61 (.38)	.53 (.28)	.32 (.10)	.32 (.10)
Effluent	159	.71 (.51)	.47 (.22)	.34 (.11)	.33 (.11)

In all 294 relevant samples a greater proportion of variation in BOD_5 is accounted for by variation in the T_1 and T_2 peaks (R^2 = .82 and .71 respectively) than the humic-like C and A peaks (R^2 = .59 and .51 respectively) and the relationship is always strongest between BOD_5 and T_1. Table 3.4 also illustrates that the relationship between BOD_5 and T_1 is not strengthened by dividing the data into surface water and effluent subsets. For all fluorophore/ BOD correlations between full dataset (n=294) and surface waters (n=135) and full dataset (n=294) and effluents (n=159) the probability of a significant correlation is 0. The correlation between fluorescence

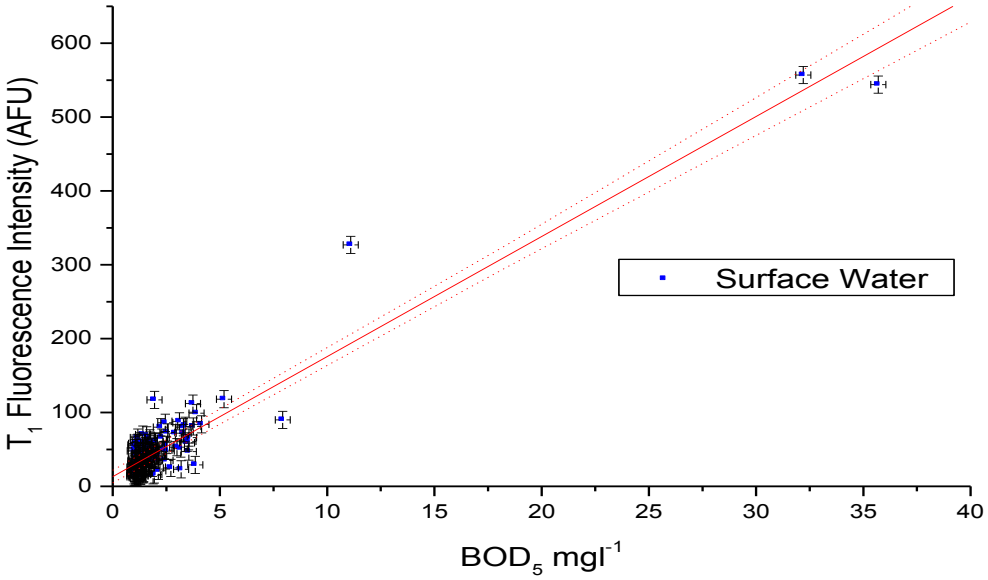
parameters and BOD_5 for the 2 subsets are statistically similar, although to a varying degree with only humic-like peaks C and A demonstrating a high probability of a relationship between fluorescence and BOD_5 (surface water/ effluent correlation probabilities (*p*) T_1 = .13, T_2 = .50, C = .85 and A = .93. However, the purpose of this chapter is to determine whether the tryptophan-like fluorescence regions (particularly that targeted by the SMF-2 fluorimeter) are good proxies for the BOD_5 test so I will concentrate on the T_1/ BOD_5 relationship for the remainder of this chapter.

Figure 3.1 shows plots of the T_1/ BOD_5 data in all relevant samples for a) full dataset b) surface waters and c) effluents. The effluent subset correlation is influenced by a number of samples which demonstrate the presence of organic material with high fluorescence intensity and low biodegradability (BOD_5). These samples may be better investigated using the Chemical Oxygen Demand (COD) test and a linear correlation may not be appropriate. The freshwater dataset is influenced by a number of high BOD_5 high fluorescence intensity samples which are likely to indicate the presence of an organic load from a pollution incident e.g. sewer discharge or agricultural pollution event. Regression lines are shown, with 95% confidence bands.

a).


T1 Fluorescence Intensity (AFU)
2500
2000
1500
1000
500
0
0
100
200
300
400
500
BOD5 mgl-1
Surface Water
Effluent

b).


T1 Fluorescence Intensity (AFU)
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
BOD5 mgl-1
Surface Water

c).

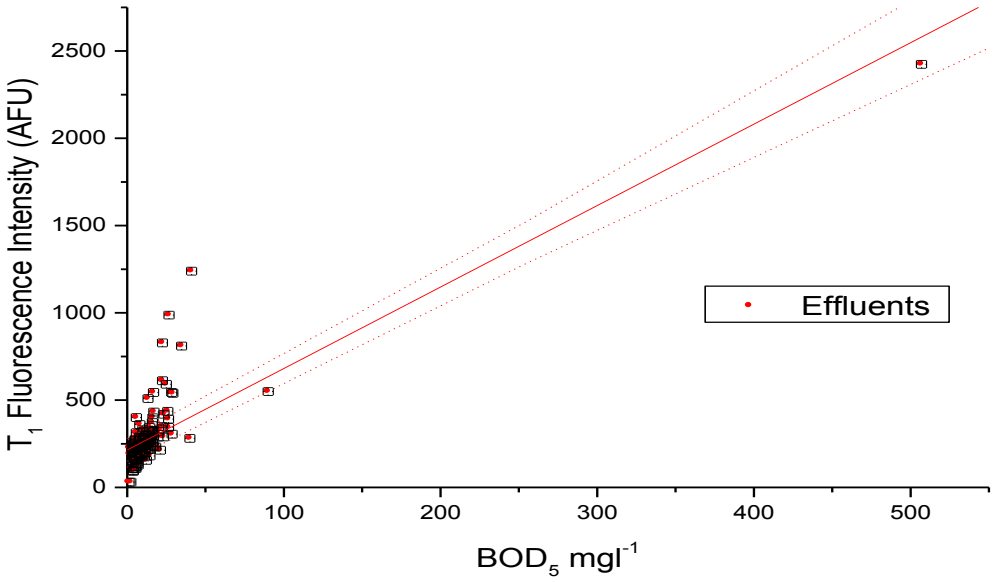


Figure 3.1: Plots of BOD_5 vs. T_1 for a) full dataset, b) surface waters and c) effluents also illustrating the standard error of the mean (as error bars) for both T_1 fluorescence intensity and BOD_5, regression lines and 95% confidence limits (on plots b and c - dotted lines). The data is clearly influenced by some high BOD_5 samples. Plot a) has not been adjusted to more clearly show all data (as plots b and c have), however, in this form it clearly illustrates the difference in gradient of the regression lines for each dataset.

The equations for the regression lines are shown in Table 3.5.

Regression Analysis

Regression analysis shows that for every 1 mgl^{-1} increase in BOD_5 concentration T_1 increases by 5.33 (+/- 0.25) AFU in the total dataset, 16.25 (+/- 0.46) AFU in the Surface Water dataset and 4.67 (+/- 0.25) AFU in Effluents, the equations of the regression lines are shown in Table 3.5. As these are all positive values this would suggest that the minimum reporting value for BOD_5 using T_1 as a proxy would be lower than the $1mgl^{-1}$ minimum

reported value quoted by the Environment Agency for the BOD_5 (ATU) test (as $0mgl^{-1}$ BOD_5 would correspond with 5.33 AFU in the full dataset, 16.25 AFU in surface waters and 4.67 AFU in effluents, plus or minus the error).

Table 3.5: New sample means and standard deviations, standard error of the mean (S.E), correlation coefficients (r = Spearman's Rho), R^2 values (in parentheses) and regression equations for the full data set, surface waters only and effluents only after exclusion of outliers (samples exceeding a z-score of 3.29) for T_1 or BOD_5.

				Including Outliers					Excluding Outliers z>3.29	
Data set	N	Mean and SD	S.E	r and R^2	Regression equation	Excluded samples	Mean and SD	S.E	r and R^2	Regression equation
All Samples	294	T_1 176.3 (+/-208.2) BOD 9.0 (+/-30.5)	T_1 12.14 BOD_5 1.78	.90 (.82)	Y=5.33(+/-0.25)X+128.49(+/-7.92)	707325 761128 761077	T_1 162.2 (+/-141.5) BOD 7.1 (+/-8.7)	T_1 8.29 BOD_5 0.51	.90 (.81)	Y=12.69(+/-0.61)*X+72.25(+/-6.77)
Surface Water	135	T_1 51.6 (+/-70.1) BOD 2.4 (+/-4.1)	T_1 11.62 BOD_5 0.35	.61 (.38)	Y=16.25(+/-0.46)X+13.21(+/-2.17)	715918 694523 771898	T_1 42.0 (+/-23.3) BOD 1.8 (+/-1.0)	T_1 2.03 BOD_5 0.09	.63 (.40)	Y=14.84(+/-1.59)*X+15.00(+/-3.29)
Effluent	159	T_1 282.2 (+/-227.3) BOD 14.6 (+/-40.5)	T_1 22.38 BOD_5 3.21	.71 (.51)	Y=4.67(+/-0.25)X+214.16(+/-10.67)	761128 761077	T_1 262.5 (+/-129.0) BOD 11.3 (+/-9.5)	T_1 10.38 BOD_5 0.09	.62 (.38)	Y=8.43(+/-0.85)*X+167.45(+/-12.58)

Figures 3.1 a, b and c show that the data are influenced by outliers. The data for outliers has been verified (to ensure no typographic errors) and a decision made to include the data in the correlation as, although they exert excessive influence upon the regression, they are representative of samples in the natural system.

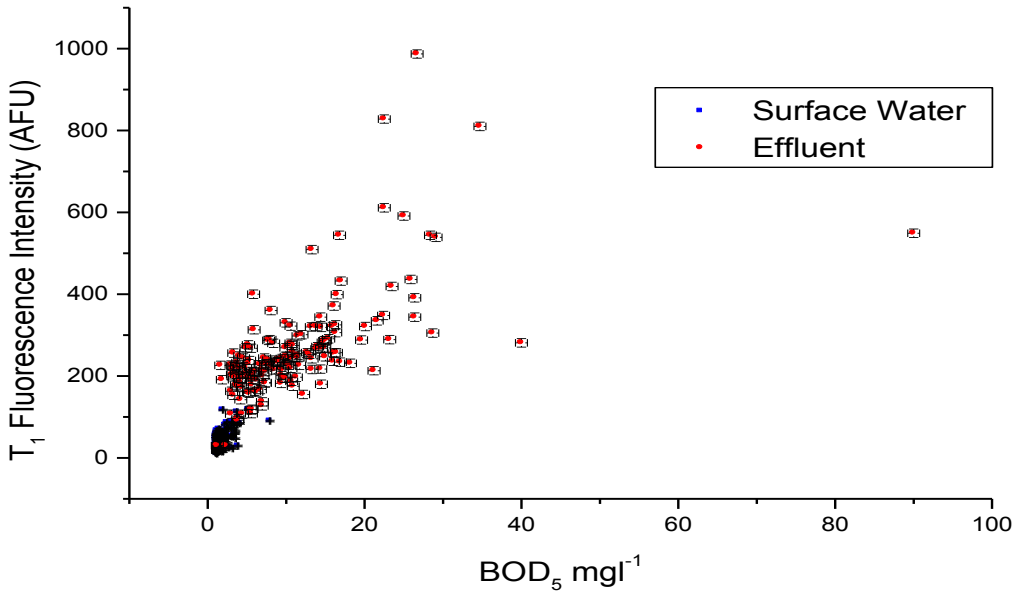
To investigate the effect of outliers samples with a z-score of greater than 3.29 were excluded. This cut-off value should exclude the most extreme 0.01% of the data. When these values are excluded from analysis the correlation coefficients and regression analyses are as illustrated in Table 3.5.

When the most extreme 0.1% outliers are excluded the gradient of the line decreases for the full dataset and effluent samples indicating a smaller increase in T_1 concentration per mgl^{-1} BOD, and the error values increase suggesting that the model becomes less accurate. This indicates that the strength of the relationship observed is strongly influenced by the outlying data. While these samples are relevant in the analysis of natural samples, in which a range of sample characters will be observed, the reliance of the relationship upon “extreme” samples suggests that within these models T_1 is not a strong proxy for BOD_5.

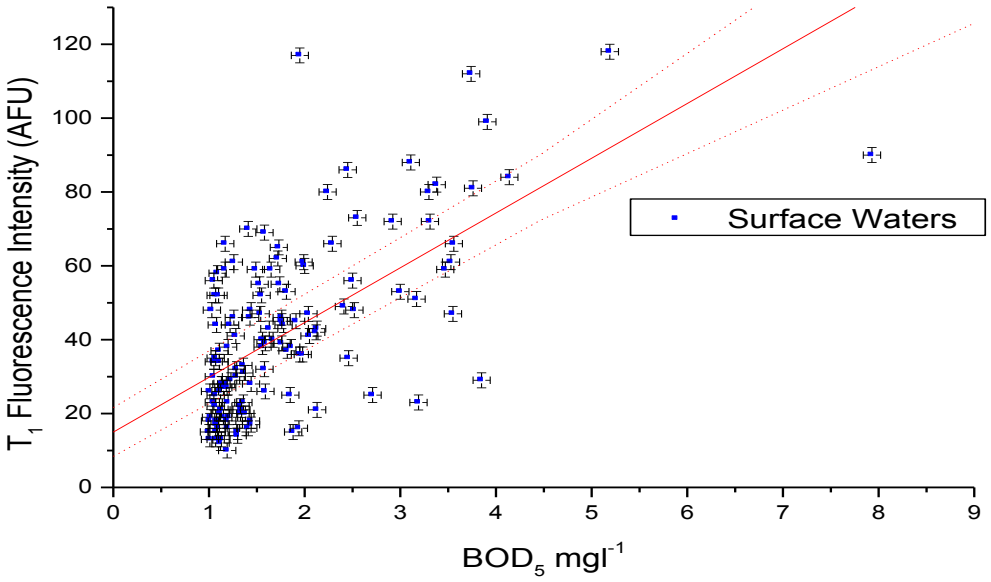
Figure 3.2 illustrates the relationship between T_1 and BOD_5 in a) all samples, b) surface waters and c) effluents when those samples classed as outliers (exceeding z=3.29) are excluded.

Correlation coefficients, R^2 va'ues and regression equations are presented in Table 3.6 for the full dataset, surface water and effluent datasets for revised populations after exclusion of outliers which are classified by a z-score of 2.58 (excludes extreme 1% of data) and 1.96 (excludes extreme 5% of data) for T_1 or BOD_5.

a.



b.



c.

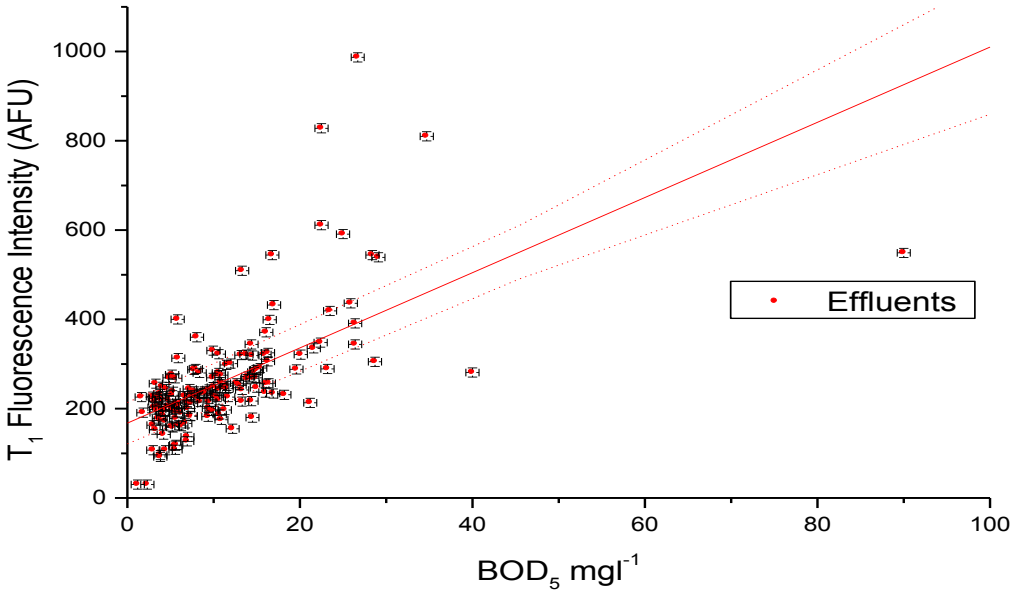


Figure 3.2: Plots of the relationship between BOD_5 and T_1 for a) full dataset, b) surface waters and c) effluents also illustrating the standard error of the mean (as error bars) for both T_1 fluorescence intensity and BOD_5, regression lines and 95% confidence limits (dotted lines) after exclusion of outliers classed as samples for which $z>3.29$ for T_1 or BOD_5.

Table 3.6: New sample means and standard deviations, standard error of the mean (S.E), correlation coefficients (r = Spearman's Rho), R^2 values (in parentheses) and regression equations for the full data set, surface waters only and effluents only after exclusion of further outliers (samples exceeding a z-score of 2.58 (excludes extreme 1% of data) and 1.96 (excludes extreme 5% of data) for T_1 or BOD_5, to determine the reliance of the model upon extreme data.

	Excluding Outliers z>2.58					Excluding Outliers z>1.96				
Data set	Excluded samples	Mean and SD	S.E	r and R^2	Regression equation	Excluded samples	Mean and SD	S.E	r and R^2	Regression equation
All Samples	719527 685865 725823	T_1 156.3 (+/-129.1) BOD 6.7 (+/- 6.9)	T_1 7.59 BOD_5 0.41	.90 (.81)	Y=15.56(+/-0.60)X+52.80(+/-5.80)	719490 761171	T_1 153.2 (+/-124.0) BOD 6.5 (+/-6.8)	T_1 7.33 BOD_5 0.40	.90 (.80)	Y=15.10(+/-0.61)X+54.55(+/-5.70)
Surface Water	n/a	n/a		n/a	n/a	n/a	n/a		n/a	n/a
Effluent	707325	T_1 257.9 (+/-115.5) BOD 11.2 (+/-9.5)	T_1 9.25 BOD_5 0.76	.70 (.48)	Y=7.76(+/-0.76)X+171.09(+/-11.11)	685865 725823	T_1 250.6 (+/-96.7) BOD 11.0 (+/-9.3)	T_1 7.79 BOD_5 0.75	.68 (.46)	Y=6.67(+/-0.65)X+177.49(+/-9.30)

The correlation is not strengthened by excluding outliers and the slope of the regression line remains relatively constant for each data set suggesting that the amount of BOD_5 data accounted for by T_1 fluorescence intensity does not increase with exclusion of outliers. However, assessment of the F-ratio indicates that, although F-ratio values are still significant at the $p<.001$ level removing the extreme 0.1% outliers makes the model less powerful, as the F-ratio reduces (indicating a greater proportion residual mean squares to model mean squares) particularly in the surface water dataset. This is shown in Table 3.7 in which the F-ratio is shown for each data sub set with varying outliers excluded.

Table 3.7: F-ratios (sum of model mean squares to sum of residual mean squares) and significance values for each dataset with varying degrees of outlier removal – the extreme 0.1% of data, the extreme 1% of data and the extreme 5% data. This indicates that exclusion of outliers makes little difference to the model for the full dataset but diminishes the model for the surface water and effluent datasets.

Dataset	All Samples		Excluding $z>3.29$ (0.1%)		Excluding $z>2.58$ (1%)		Excluding $z>1.96$ (5%)	
	F-ratio	Sig (p)	F-ratio	Sig (p)	F-ratio	Sig (p)	F-ratio	Sig (p)
All Samples	455.6	<.001	438.4	<.001	663.2	<.001	623.3	<.001
Surface Water	1243.4	<.001	87.4	<.001	n/a	n/a	n/a	n/a
Effluent	352.3	<.001	97.4	<.001	104.4	<.001	105.7	<.001

The ability of T_1 fluorescence intensity to predict BOD_5 concentration, in surface waters in particular, is strongly influenced by outlying data points.

For each regression the F-ratio is significant (p <0.001) indicating that T_1 predicts BOD_5 significantly well. However, as the slopes of the regression

lines for surface waters and effluents are very different the relationship between BOD_5 and T_1 for the two datasets is different, thus it is not possible to use the same model to predict BOD_5 using T_1 for surface waters and effluents. As the intercept value (on the T_1 axis) is positive it should be possible to predict BOD to $<1mgl^{-1}$ the minimum reporting value for the Environment Agency analysis. However, it may be seen in the plotted data that a wide distribution of T_1 values is associated with a single BOD_5 value (which has an error of +/- c.8%). 95% confidence limits for the T_1/ BOD_5 correlations are as follows when all outliers are included:

Full dataset: .88 to .92

Surface Waters: .49 to .71

Effluents: .62 to .78

When outliers (as defined by a z-score exceeding 3.29 for either T_1 or BOD_5) are excluded the 95% confidence limits do not improve:

Full dataset: .88 to .92

Surface Waters: .52 to .72

Effluents: .51 to .71

These broad 95% confidence limits, in conjunction with the R_2 values and regression analyses suggest that fluorescence spectroscopy may be an adequate indicator of BOD_5 when the type of sample is unknown and the data is assessed with the full dataset model above in which 82% of BOD_5 data is accounted for by T_1fluorescence intensity. However, it is not a strong proxy for BOD_5 when the surface water or effluent models are applied in which only

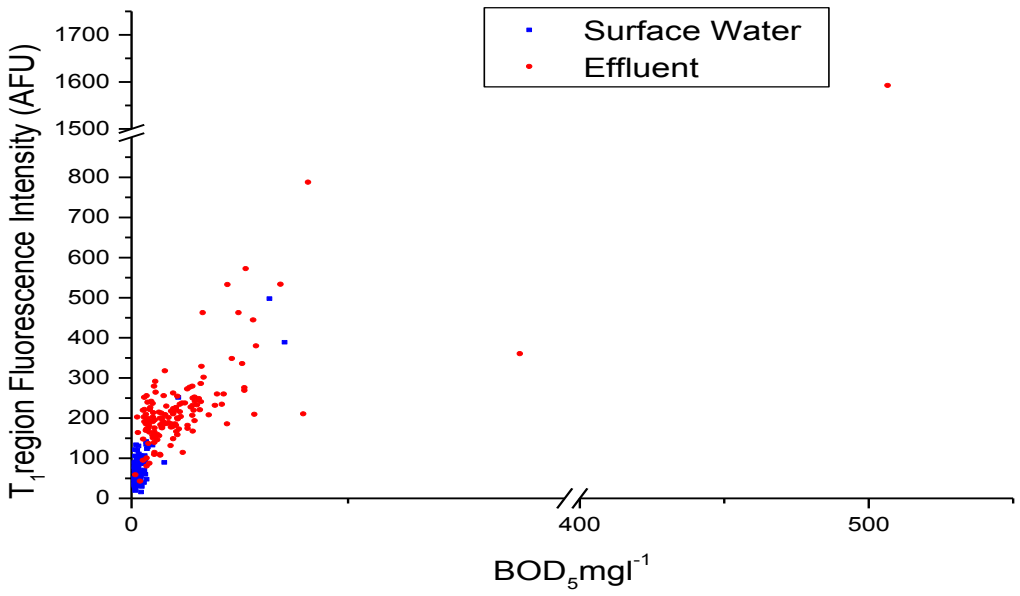
38% and 51% (respectively) of BOD_5 data is accounted for by T_1 fluorescence intensity.

3.3.4. Relationship between Fluorescence Intensity Measured on SMF-2 Fluorimeter and BOD_5

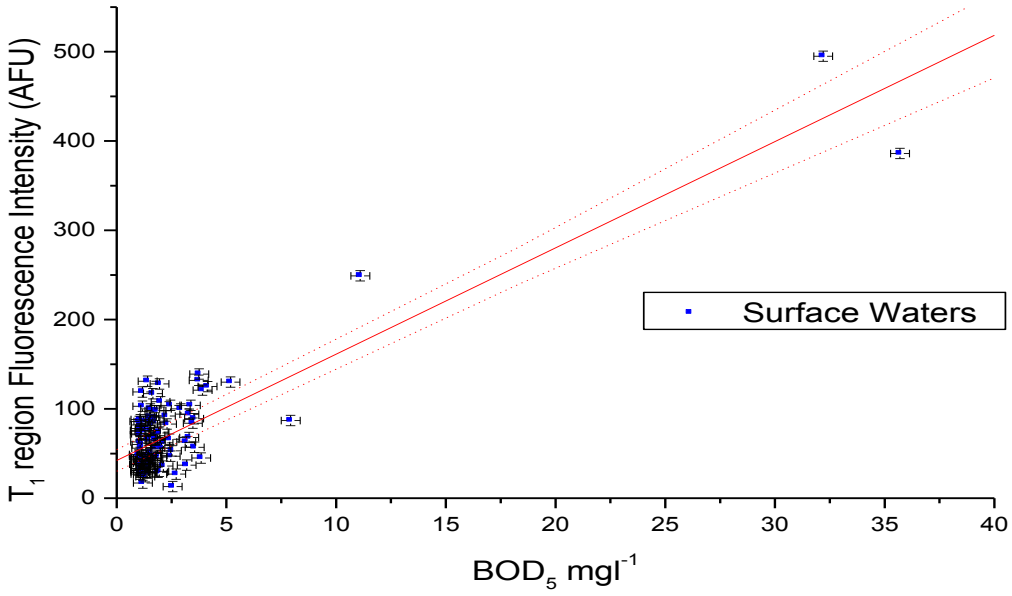
The samples analysed using the Cary Eclipse were also analysed for fluorescence using an SMF-2 portable fluorimeter (Safe Training Systems, U.K). The SMF-2 uses a xenon flash light lamp and targets the T_1 peak as described in section 2.1.1. Comparable SMF-2 fluorescence and BOD_5 data is available for 249 of the 294 samples, of which 111 are surface waters and 138 are effluents.

When correlated with BOD_5 values the SMF-2 T_1 values display a good relationship for the total dataset ($r = .84$, $R^2 = .71$), but, like the Cary Eclipse results, was less convincing when the data was split into sample types with surface waters ($r = .41$, $R^2 = .17$) and effluents ($r = .61$, $R^2 = .37$) respectively. Figure 3.3 illustrates fluorescence intensity in the T_1 region as measured on the SMF-2 fluorimeter plotted against BOD as reported by the Environment Agency for a) all samples, b) Surface Waters and c) Effluents.

a).


Surface Water
Effluent
T1region Fluorescence Intensity (AFU)
1700
1600
1500
800
700
600
500
400
300
200
100
0
0
400
500
BOD5mgl-1

b).


Surface Waters
T1 region Fluorescence Intensity (AFU)
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
BOD5 mgl-1

c).

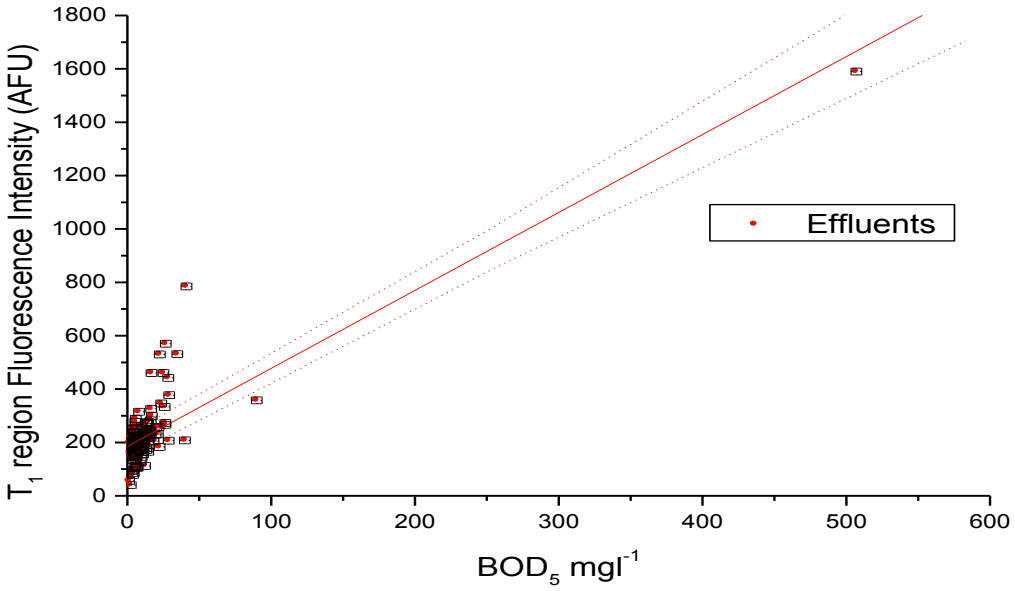


Figure 3.3: Plots showing the relationship between T_1 region fluorescence intensity measured on SMF-2 and BOD_5 for a) full dataset, b) surface waters and c) effluents with standard error of the mean for T_1 region fluorescence and BOD_5 as error bars, regression lines and 95% confidence limits (dotted lines) for surface water and effluent subsets.

The linear regression equations are as follows:

Full dataset Y=3.34(+/-0.18)X+127.85(+/-6.01), F-ratio=361.65, p <0.001.

Surface Waters Y=11.91(+/-0.64)X+42.23(+/-3.27), F-ratio=350.00, p <0.001

Effluents Y=2.92(+/-0.16)X+185.66(+/-7.44), F-ratio = 322.82, p <0.001.

The F-ratios show that in all cases the model is a good fit for the data. In all cases the increase in T_1region fluorescence intensity per mgl^{-1}BOD is low compared with the increase in T_1 recorded on the Cary-Eclipse. This indicates lower sensitivity in the SMF-2 fluorescence analysis than the Cary-Eclipse,

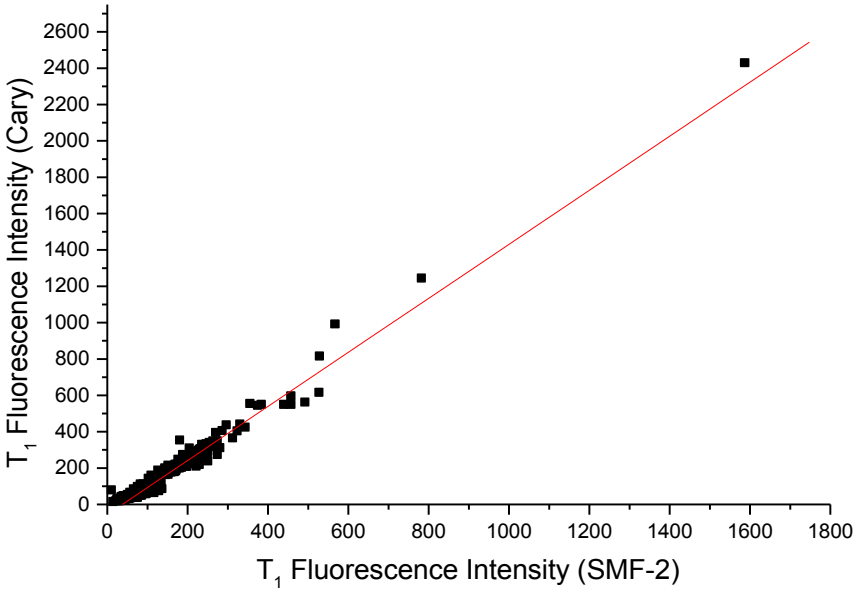
which is to be expected as a result of the sophistication of the light source of the bench instrument as opposed to the portable.

3.3.5. Agreement between the Cary Eclipse and SMF-2 Fluorescence Spectrophotometers

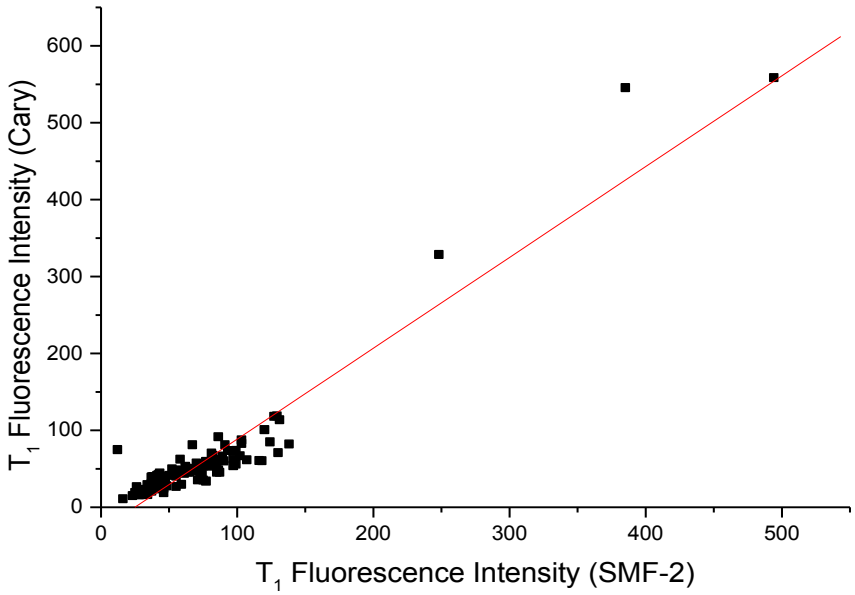
Fluorescence intensity measured on the SMF-2 correlates well with the intensity measured using the Cary spectrophotometer ($r = .97$, $R^2 = .94$) for the total dataset (Figure 3.4a) which is in line with the relationship found by Baker et al., (2004) of $r = .91$ ($R^2 = .83$). Also surface water samples (Figure 3.4b) ($r = .87$, $R^2 = .76$) and effluents (Figure 3.4c) ($r = .93$, $R^2 = .87$). The mean ratio of SMF-2 value to Cary Eclipse T_1 value is 0.99 (s.d. 0.42) and comparable SMF-2 and Cary Eclipse values were recorded for 249 samples in total. The mean ratio for surface waters is 0.74 (s.d. 0.51) n = 111 and effluents is 1.18 (s.d. 0.17) n = 138.

There is good agreement between the values measured on the two instruments. The difference in the ratio between SMF-2 and Cary Eclipse fluorescence values indicates that, as the ratio is low with high standard deviation for surface waters but strong for effluents, the SMF-2 has lower operational sensitivity than the Cary Eclipse. However, both instruments are suitable for the measurement of T_1 fluorescence. The Cary Eclipse is likely to be more accurate and as it is large and not portable it is a suitable laboratory based instrument. The SMF-2 is fit for purpose as a field instrument.

a).


T1 Fluorescence Intensity (Cary)
T1 Fluorescence Intensity (SMF-2)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600

b).


T1 Fluorescence Intensity (Cary)
T1 Fluorescence Intensity (SMF-2)
0
100
200
300
400
500
600

c).

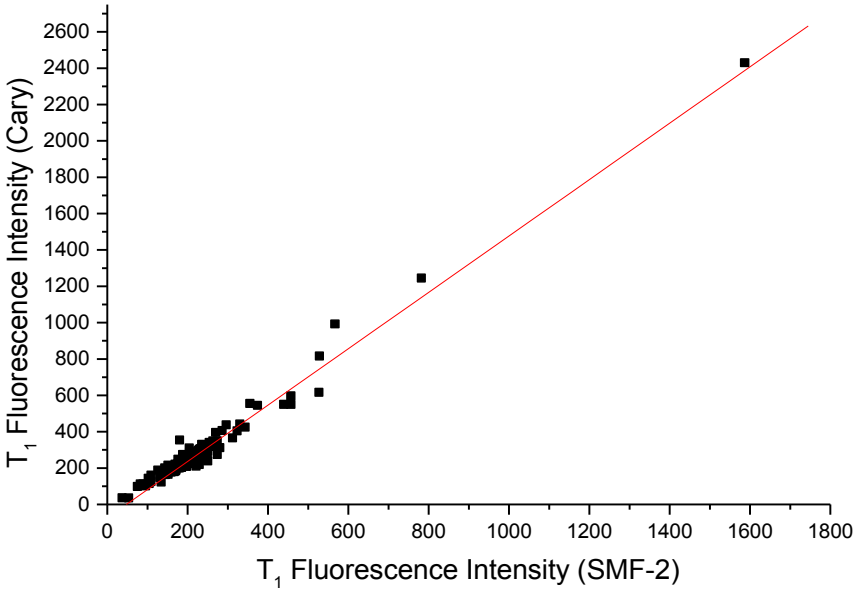


Figure 3.4: Plots showing the relationship between peak T_1 fluorescence intensity measured on the Cary Eclipse and T_1 region fluorescence intensity measured on the SMF-2 for a) total dataset, b) surface waters and c) effluents

Equations for the regression lines for T_1 region measured on the Cary-Eclipse vs. T_1 measured on the SMF-2 are as follows:

Full data set (Y=1.49(+/-0.02)X+-55.73 (+/-3.44)), F-ratio = 8535, p <0.001.

Surface waters (Y=1.18(+/-0.04)X+-29.44(+/-3.50)), F-ratio = 1016., p <0.001

Effluents (Y=1.55(+/-0.02)X+-73.75(+/-6.06)), F-ratio = 4932., p <0.001.

Generally the linear regressions fit the data well with low residual mean squares compared with the model mean squares, and as F-ratio values are high and significant the model may be classed as a good representation of the relationship between T_1 measured on the Cary-Eclipse and SMF-2 fluorimeter.

It may be summarised that for each unit increase in fluorescence intensity measured by the SMF-2 one sees an increase of 1.49 units (all samples), 1.18 units (surface waters) and 1.55 units (effluents) suggesting that the output of the two instruments are more similar when measuring “cleaner” surface water samples.

3.4. Assessment of the Importance of Other Factors Influencing the Fluorescence/ BOD_5 Relationship

3.4.1. Time Elapsed between Sample Collection and Analysis

This section presents an assessment of the change in the quality of the T_1/ BOD_5 relationship with time between sample collection and analysis. Samples are excluded from this section if the time between sample collection by the Environment Agency and fluorescence analysis exceeded 72 hours which allows time for collection, transportation and analysis of samples and excludes time during which sample storage protocols may not have been followed. Once again samples are presented as total data set, surface waters and sewage effluents. The total number of samples is reduced to 266, of which 127 are surface waters and 139 effluents.

Table 3.8 illustrates the original correlations for the full dataset of 294 samples, comprising 135 surface waters and 159 effluents, between fluorescence as measured on the Cary-Eclipse, and BOD_5 as listed in section

3.3.3. Also presented are the correlation coefficients derived when samples analysed more than 72 hours after collection are excluded.

Table 3.8: Correlations (Spearman's Rho) and R^2 values (in parentheses) for relationships between the four common fluorescence peaks T_1, T_2, C and A (measured on the Cary-Eclipse spectrophotometer) and BOD_5 in all samples, surface water and effluent subsets for the 294 samples previously discussed and a reduced sample set of 266 samples which include only those analysed for fluorescence within 72 hours of sample collection. Significant correlations at the 0.01 level (2-tailed) are highlighted.

	N	Peak T_1	Peak T_2	Peak C	Peak A
All Samples	294	.90 (.81)	.84 (.71)	.77 (.59)	.72 (.52)
Surface water	135	.61 (.37)	.53 (.28)	.32 (.10)	.32 (.10)
Effluent	159	.71 (.50)	.47 (.22)	.34 (.12)	.33 (.11)
Excluding >72 hours					
All Samples	266	.91 (.83)	.85 (.72)	.78 (.61)	.74 (.55)
Surface water	127	.62 (.38)	.53 (.28)	.32 (.10)	.33 (.11)
Effluent	139	.73 (.53)	.52 (.27)	.32 (.10)	.34 (.12)

This analysis indicates that the amount of BOD_5 data accounted for by fluorescence data in surface waters samples are not significantly affected by time from sampling with probabilities of a significant similarity being (*p)* T_1= .90, T_2= 1.0, C=1.0 and A= .93. Effluents are more variable with the probability of a significant relationship being only moderate in the more bioavailable T_1 and T_2 peak with probabilities of significantly similar correlations (*p*) T_1= .73, T_2= .58, C= .85 and A= .92 while the full dataset is

influenced by the effluent samples with probabilities (*p*) T_1= .52, T_2= .68, C= .77 and A= .62.

Analysis of the change in regression equation between samples analysed within 72 hours of collection and more than 72 hours after collection shows that the F-ratio (ratio of model mean squares to residual mean squares) decreases in peaks T_1 and T_2 the sample groups analysed more than 72 hours after collection, indicating a worse fit for the model suggesting increased heterogeneity of samples. Peak C and A display a more varied range of responses. Further, the gradient of the regression line is similar in samples analysed within and without 72 hours from sample collection, except surface water samples in which the gradient for peaks T_1 and T_2 are greater in samples analysed more than 72 hours after sample collection. This seems to indicate an evolution of fluorescence with time leading to increased fluorescence intensity per mgl^{-1} BOD_5. Equations if the regression lines and F-ratios are illustrated in Table 3.9.

Table 3.9: Regression equations and F-ratios (with significance in parentheses) for relationship between all fluorescence peaks (T_1, T_2, C and A) and BOD_5 comparing samples analysed for fluorescence intensity within 72 hours of collection and those analysed more than 72 hours after sample collection. In the "All Samples" and "Effluent" groups these is a clear increase in the residual mean squares value causing a decrease in the quality of the models ability to predict $BOD_5$from fluorescence intensity over time, except humic like peaks C and A in "Surface waters" for which the model improves with time from sampling (as seen by an increase in F-ratio – decrease In residual mean squares proportional to model mean squares).

		Peak T_1		Peak T_2		Peak C		Peak A	
		Regression equation	F-ratio (sig.)	Regression equation	F-ratio (sig.)	Regression equation	F-ratio (sig.)	Regression equation	F-ratio (sig.)
All Samples	<72 hours	Y=5.33(+/-0.25)X+128.49(+/-7.92)	455.69 (<.0001)	Y=15.33(+/-0.64)X+215.06(+/-20.39)	568.97 (<.0001)	Y=2.05(+/-0.26)X+202.06(+/-8.36)	60.73 (<.0001)	Y=9.94(+/-0.42)X+267.86(+/-13.29)	563.06 (<.0001)
	>72 hours	Y=5.85(+/-0.31)X+122.41(+/-9.21)	355.79 (<.0001)	Y=17.19(+/-1.02)X+219.26(+/-30.35)	283.30 (<.0001)	Y=2.36(+/-0.27)X+205.77(+/-7.90)	78.95 (<.0001)	Y=10.59(+/-0.53)X+286.53(+/-15.72)	400.31 (<.0001)
Surface Waters	<72 hours	Y=16.25(+/-0.46)X+13.21(+/-2.17)	1234.21 (<.0001)	Y=40.44(+/-0.98)X+35.74(+/-4.62)	1701.65 (<.0001)	Y=14.78(+/-1.53)X+71.71(+/-7.21)	93.51 (<.0001)	Y=42.76(+/-2.82)X+116.04(+/-13.3)	229.78 (<.0001)
	>72 hours	Y=28.69(+/-1.08)X+-11.52(+/-6.08)	711.75 (<.0001)	Y=93.19(+/-4.34)X+-73.93(+/-24.54)	461.55 (<.0001)	Y=14.98(+/-1.18)X+91.62(+/-6.66)	161.76 (<.0001)	Y=49.47(+/-2.10)X+132.83(+/-11.9)	553.02 (<.0001)
Effluents	<72 hours	Y=4.67(+/-0.25)X+214.16(+/-10.67)	352.33 (<.0001)	Y=14.35(+/-0.82)X+331.31(+/-35.15)	306.9 (<.0001)	Y=1.3(+/-0.21)X+298.12(+/-9.02)	38.49 (<.0001)	Y=9.19(+/-0.43)X+342.01(+/-18.59)	449.69 (<.0001)
	>72 hours	Y=6.89(+/-0.72)X+177.07(+/-11.84)	91.00 (<.0001)	Y=15.60(+/-2.45)X+288.89(+/-40.18)	40.50 (<.0001)	Y=3.07(+/-0.69)X+273.21(+/-11.34)	19.73 (<.0001)	Y=6.30(+/-1.36)X+360.82(+/-22.27)	21.50 (<.0001)

This analysis addresses degree of sample change during storage before fluorescence analysis but does not address sample degradation before BOD_5 analysis. The time lapse between sample collection and BOD_5 analysis by the Environment Agency is an unknown and is therefore assumed to be constant on the basis that sample storage at the Environment Agency should be standardised and such that sample degradation is minimised.

3.4.2. Analysis of Site, Catchment and Regional Scale Relationships

Site Scale Analysis

Only one site was sampled enough times to provide data for a correlation, so it is not possible to draw any conclusions about the nature of the BOD_5/ fluorescence correlation on a site by site basis.

Catchment Scale Analysis

Ultimately it is not possible to assess the quality of the fluorescence/ BOD_5 relationship on a river reach basis as only two reaches reported sufficient numerical BOD_5 values to enable correlation with fluorescence results. In the majority of cases BOD_5 was simply reported as a “>” value and, thus, is unsuitable for correlation. The two reaches that were suitable showed that the relationship is not improved by consideration on a river reach basis. The correlation coefficients returned for the two suitable reaches are shown in Table 3.10.

Table 3.10: Number of samples from catchment and correlation coefficients (Spearman's Rho) and R^2 values (in parenthesis) between fluorescence parameters and BOD_5 in surface water s from catchments in which more than 2 surface water samples were collected for analysis over the course of the analytical period. Significant correlations at the 0.05 level (2-tailed) at least are highlighted.

Catchment	**N**	**Correlation with BOD_5 (Spearman's rho)**			
		T_1	T_2	C	A
6	3	-.50 (.25)	-1.0 (1)	-.50 (.25)	-.50 (.25)
9	5	-.90 (.81)	-.30 (.09)	-.72 (.52)	-.72 (.52)
17	4	-1.0 (1)	-1.0 (1)	-.80 (.64)	-1.0 (1)
18	3	.50 (.25)	.50 (.25)	-.50 (.25)	-1.0 (1)
21	5	.36 (.13)	.21 (.04)	.05 (.00)	.05 (.00)
23	5	.67 (.45)	.98 (.96)	.10 (.01)	.30 (.09)
33	5	.30 (.09)	.36 (.13)	-.60 (.36)	.80 (.64)
45	5	.60 (.36)	.20 (.04)	-.10 (.01)	-.10 (.01)
49	7	.71 (.50)	.75 (.56)	.57 (.33)	.71 (.50)
52	6	.03 (.00)	.89 (.79)	.89 (.79)	.77 (.59)
57	4	.63 (.40)	.63 (.40)	.32 (.10)	.32 (.10)

There are insufficient significant correlations to indicate a consistent improvement or decline in the relationships between fluorescence parameters and BOD_5 on a catchment scale.

Regional Scale Analysis

Assessing the importance of regional sample relativity Table 3.11 illustrates the change in relationship between fluorescence parameters and BOD_5 for all samples which returned a BOD_5 value (n = 362), not simply those which are classed as being in the South West of England.

Table 3.11: Analysis of the influence of geography upon the correlation between T_1, T_2, C and A fluorescence intensities and BOD_5 for full dataset, surface water and effluent subsets sub sets in all samples collected, not only those from South West England. Numbers of samples, correlation coefficients (Spearman's Rho) and R^2 values (in parentheses) are presented. Correlation coefficients significant at the 0.01 level (2-tailed) are highlighted.

	N	Peak T_1	Peak T_2	Peak C	Peak A
All Samples	294	.90 (.81)	.84 (.71)	.77 (.59)	.72 (.51)
Surface water	135	.61 (.38)	.53 (.28)	.32 (.10)	.32 (.10)
Effluent	159	.71 (.51)	.47 (.22)	.34 (.11)	.33 (.11)
From any region					
All samples	362	.87 (.76)	.84 (.71)	.75 (.56)	.67 (.45)
Surface water	182	.62 (.38)	.54 (.29)	.38 (.14)	.37 (.14)
Effluent	175	.66 (.44)	.48 (.23)	.28 (.08)	.29 (.08)

There is no clear geographical relativity in the relationship as samples from a defined geographical region return statistically unsimilar correlation coefficients to those from a more diverse geographical distribution. The probability of correlation coefficients being statistically similar regardless of sample location is low for all peaks except T_2 in which the probability of a

statistically similar correlation is high in all water types (all samples p=1, surface waters p=.91 and effluents p=.91).

3.5. Discussion

3.5.1. Comparison with Previous Work

This study incorporates a greater number of samples with paired BOD_5 and fluorescence data, with greater geographical diversity than previous work on the subject. Despite the great variation inherent in this data set a good correlation between fluorescence and BOD_5 is demonstrated for the T_1 peak. The relationship found in this study is clearly of similar significance to previous studies, despite the large number of samples, the variety of sample types studied and their geographical distribution.

In the published paper from this chapter (Hudson et al., (2008) geographical relationships are investigated using Geographically Weighted Regression (GWR). This technique is not addressed in this thesis as the work for the paper was undertaken by Prof. Chris Brunsdon, University of Leicester.

3.5.2. Factors that Influence the BOD_5/ Fluorescence Relationship

Sample Character Variability

To demonstrate a strong correlation between T_1 fluorescence and BOD_5 for a large dataset of such geographically diverse samples it is necessary to include both the high and low end members of the dataset and outlying

samples. In subdividing the data into sample types the relationship, although statistically significant, begins to decrease as site specific factors, microbial community and BOD_5 method error (around 8% calculated from data in HMSO, 1988) begin to exert an influence. An improvement in the relationship after splitting the dataset into surface waters and effluents would suggest homogeneity of samples from site to site. However, as this work represents a natural system from a range of geographical locations and seasons there is enormous character variability in both the surface water and effluent datasets so correlations do not improve. Surface waters are subject to geological and climatic variation, agricultural and anthropogenic inputs whilst wastewater effluents are subject to variations in character determined by treatment specific impacts.

Microbiological Influences on the T_1 / BOD_5 Relationship

Microbial activity, measured by oxygen depletion in the BOD_5 test, is thought to relate to fluorescence peak T, either because peak T is present in a bioavailable substrate, or because peak T is produced by microbial action. Cammack et al., (2004) proposed that observation of the T peaks is actually an observation of the balance of decline in substrate and increase in community. Elliott et al., (2006) show that the T peaks intensities increase with colony forming units for a riverine microbial community. Interestingly, strong correlations have also been found between peak T fluorescence and Chemical Oxygen Demand (COD) which measures the total oxidisable material present (Lee and Ahn, 2004). In contrast, humic-like material relates

less strongly to the BOD_5 test, indicating that it may be less readily available to the bacterial community and less easily biodegraded.

Low BOD_5 Samples

The relationship between BOD_5 and fluorescence for very low BOD_5 samples is difficult to determine in this work as the Minimum Reported Value of BOD_5 is $1mgl^{-1}$ and values lower than this are reported as "<1". In this instance the study of fluorescence EEMs is more informative of the nature of the organic material than BOD_5, giving an indication of the types of material present (humic-like or "fresh" microbial derived) and the relative proportions of each type. In addition, analysis of the T_1 peak fluorescence intensities which correlate well with the biodegradable fraction of organic matter may develop an understanding of the proportion of more bioavailable material present, to very low concentrations, and the potential oxygen depleting potential of this load in natural waters.

3.6. Summary

1. Fluorescence spectroscopy has the potential to be a powerful laboratory or field tool in the measurement and characterisation of bioavailable organic matter in water, with the potential to predict BOD_5values to less than the $1.0mgl^{-1}$ MRV of the Environment Agency with particular application in effluent quality monitoring, pollution event investigation and regular on-site testing. The SMF-2 is capable of field

measurement of BOD_5 equivalent. However, confidence in such predictions is low due to the scatter of data observed in this work.

2. To improve the accuracy of fluorescence as a predictive tool for BOD_5 further work is required to develop a greater understanding of local sample heterogeneity, the importance of geographical/ spatial influences on sample character, and other factors which may influence the strength of the correlation.

3. Although the general predictive ability of fluorescence as a proxy for BOD_5 is poor at lower BOD concentrations, for "clean" samples fluorescence analysis could more accurately indicate of the types of organic material present (natural or anthropogenic/ labile or recaltriant), the relative proportions of these different types, and could give an understanding of the proportion of more bioavailable or labile material and its oxygen depleting potential in natural water.

4. To make the most of the technique it is necessary to consider fluorescence spectroscopy as a more accurate and flexible indicator of bioavailability than BOD_5. To maximise its potential as an analytical tool it should be "un-coupled" from BOD_5 and, instead, used as an independent indicator test for bioavailable organic matter presence, associated biological activity and oxidising potential with associated impacts on water quality.

4. RELATIONSHIPS BETWEEN FLUORESCENCE AND OTHER WATER QUALITY PARAMETERS

Rationale

An investigation into relationships between fluorescence and other common water quality analyses to determine the character of fluorescent organic matter and the potential of fluorescence spectroscopy for on-site or laboratory rapid analysis.

4.1. Introduction

A body of research exists which suggests that the tryptophan-like fluorescence of a water or wastewater may relate to its intrinsic Biochemical Oxygen Demand (BOD_5). This is supported by the work presented in Chapter 3. However, other commonly measured water quality parameters may also be indicated by the fluorescence characteristics of water and may tell us something about the properties of the fluorescent material present. The relationships with other common parameters have been less well investigated than the relationship with BOD_5, probably because other techniques often already have efficient on-site testing methods being based on colourimetry or direct measurement.

We are aware that “nutrients” in nature are digested during the respiration process by heterotrophs. The previous chapter discussed the bioavailability of fluorescent organic material and proposed that, as the correlations between

tryptophan-like fluorescence and BOD_5 are greater than correlations between humic-like material and BOD_5, tryptophan-like fluorescent material is more available to microorganisms than humic-like material. In the same way, significant correlations between tryptophan-like fluorescence and standard nutrient analysis may suggest that if nutrients are associated with the tryptophan-like material they may also be labile. Similarly if nutrient levels correlate closely with humic-like materials this may suggest binding within these less labile molecules and thus, a lower bioavailability.

Also of interest is the possibility of an on-site multi-function fluorescence tool. As previously stated in Chapter 3 tryptophan-like fluorescence correlates well with BOD_5 and thus may be used as an almost instantaneous indicator of BOD_5 in on-site conditions. This may be particularly relevant if site specific correlations can be achieved (after longer term monitoring of the BOD_5 / fluorescence relationship). Similarly, if particular fluorescence peaks relate to other common water quality parameters it may be possible to develop a multi-parameter tool which indicates other nutrient levels by analysis of fluorescence intensity at specific wavelengths.

This chapter presents correlation data between fluorescence and common water quality parameters for the same samples analysed in Chapter 4, with all fluorescence and TOC analysis carried out at the University of Birmingham, and all chemical water quality undertaken by the Environment Agency.

4.2 Literature Review

Over the last 15 years correlations have been determined between common aquatic fluorophores and a range of common water quality parameters including the majority of those cited in this work. Although a reasonable body of research exists, the work on each fluorophore and water quality parameter is thin, so few papers are cited in this literature review.

The papers cited below determine correlations between fluorescence intensity and water quality parameters in a range of aquatic media from river water (Baker, 2002a) to pollution events and highly polluted effluents (Baker et al., 2003), raw and treated sewage (Reynolds, 2002) and supernatant from the activated sludge treatment process (Tartakovsky et al., 1996). Work which relates fluorescence to water quality is summarised in Henderson et al., (2009) with the works most relevant to this thesis detailed in Table 4.1.

Table 4.1: A summary table of existing literature investigating relationships between fluorescence intensity and common water quality parameters, including the fluorophores examined, water quality parameter against which it was correlated and the authors of the works.

Fluorophore	Water quality parameter	Author
T_1 Intensity	Total Organic Carbon (TOC)	Baker, 2002a, b; Reynolds 2002; Vassel and Praet, 2002; Hudson et al., 2008
T_1 Intensity	Ammonia	Baker et al., 2003; Baker and Inverarity, 2004
T_1 Intensity	Nitrate	Baker and Inverarity, 2004; Cammack et al., 2004
T_1 Intensity	Phosphate	Baker and Inverarity, 2004; Cammack et al., 2004
T_1 Intensity	Chemical Oxygen Demand (COD)	Lee and Ahn, 2004; Reynolds, 2002; Tartakovsky et al., 1996; Vassel and Praet, 2002
T_2 Intensity	Ammonia	Baker et al., 2004; Baker and Inverarity, 2004
T_2 Intensity	COD	Lee and Ahn, 2004
C Intensity	TOC	Baker, 2002b; Clark et al., 2002; Comber et al., 1996; Newson et al., 2001
C Intensity	Ammonia	Baker et al., 2003

Chemical Oxygen Demand (COD) is a measure of organic load, determining the oxygen required to chemically oxidise all organics to CO_2 and like BOD_5 COD correlates well (r=0.96-0.97) with tryptophan-like fluorescence intensity (Lee and Ahn, 2004; Reynolds, 2002). Tartakovsky et al., (1996) also proposed excellent relationships between fluorescence parameters and COD (r=>0.9), but suggested that the fluorophore in question could be NADH. The wavelengths quoted are sufficiently close to those of T_1 (290/340 and 290/382) to suggest that this may, in fact be the T_1 peak which has been blue-

shifted in the media in question (activated sludge liquor), or that the peak is in fact NADH but heavily influenced by the closely neighbouring T_1 peak. The tryptophan-like fluorescence/ BOD_5 and tryptophan-like fluorescence/ COD relationships indicate that the more degradable fraction may constitute a highly fluorescent but low carbon fraction of the water.

Total Organic Carbon (TOC) is a measure of the total organic carbon load in water and is, therefore, an indicator of oxygen depleting potential. TOC has been found to correlate well with peak C intensity (r=0.68, Baker, 2002a; r=0.99, Clark et al., 2002) but less well with T_1 intensity (r=0.2, Baker, 2002a; r=0.62, Baker, 2002b). This supports the reasoning that peak C is an indicator of the stable, humic like carbon present in water and that changes in fluorescence intensity may reflect changes in TOC concentration.

Good correlation has been found to exist between ammonia (a nutrient formed by the breakdown of proteins and urea thus found in wastewaters, also manufactured for agricultural use) and the intensity of all fluorophores but only in the case of pollution events (r=0.96 (T_1), 0.89 (C), Baker et al., 2003) and in high strength wastes (r=0.95-0.98 (T_2), Baker et al., 2004). Since the correlation is poor under standard conditions (r=0.08, Baker et al., 2003) this suggests that it may only be relevant at high ammonia concentrations and reflect the direct relationship between ammonia and wastewaters.

Nitrate and phosphate, other nutrients found in effluents, agricultural run-off and industrial discharges have also been found to correlate well with T_1

fluorescence intensity (r=0.87 and 0.8 respectively, Baker et al., 2005). As essential contributory factors to eutrophication it is not surprising that the presence of available nitrogen has been found to promote microbial activity and the production of microbial organic matter (Wilson and Xenopoulos, 2009) which contribute to tryptophan-like fluorescence (Cammack et al., 2004, Elliott et al., 2006). Nitrogen and phosphorous species are common indicators of more “polluted” waters and are present in high concentrations in diffuse pollution from agricultural land, sewage effluents, industrial waste and other media.

4.3. Description of Samples

Results are presented for the samples discussed in Chapter 3 which were collected and analysed for a range of water quality parameters (predominantly but not exclusively from South West England) by the Environment Agency. Fluorescence and TOC analysis of sub-samples were undertaken at the University of Birmingham. In total 574 samples were analysed. All are included in this chapter and no consideration is given to geographical relationships. Fluorescence and water quality data for all 574 samples are presented in Appendix 2. The aim of this chapter is to investigate relationships between the fluorescence profile of surface waters and sewage effluents and their chemical water quality profiles as measured by the Environment Agency as part of their regulatory activities. A wide range of chemical parameters were analysed and reported and the suite of analysis undertaken depended largely upon the purpose of the test i.e. standard monitoring programme or in

response to a suspected pollution incident. For this reason, as in Chapter 4 some of the surface water samples are likely to be for the analysis of pollution incidents. However, as they are samples from surface waters and the identifying sample names provided by the Environment Agency are river or catchment based they are classified in this work as surface waters. In order to propose a meaningful argument about relationships found between fluorescence and water chemistry parameters only those parameters with values reported for a significant number of sites are included in this chapter.

The selected parameters for surface waters: Fluorescence intensity of peaks T_1, T_2, C and A measured on the Cary Eclipse; SMF-2 fluorescence output; COD; TOC; ammonia as N; nitrate as N and orthophosphate as P.

For sewage effluents the selected parameters are fluorescence intensity of peaks T_1, T_2, C, A measured on the Cary Eclipse; SMF-2 fluorescence output; COD; TOC and ammonia.

Data is presented for all samples for which fluorescence data was obtained. Any water quality values which were quoted as “>” or which were negative were excluded from correlation with the fluorescence data. For this reason the correlation coefficients quoted are for different numbers of samples depending upon the water quality parameter involved. As in Chapter 3 all fluorescence data presented is from the lowest dilution undertaken.

In this chapter correlation and regression analyses should be carried out and presented as in Chapter 3, with an appropriate number of significant figures quoted, significance testing on and between lines and slope analysis for samples analysed within 72 hours of collection and more than 72 hours from sample collection. Furthermore, multivariate techniques such as PCA could be used to test the data in this chapter further.

4.4. Relationships between Fluorescence and Chemical Water Quality Parameters

In this section correlations between fluorescence and common water quality parameters are presented. Results are presented for all samples which returned fluorescence values regardless of location. However, also presented is an assessment of the importance of the time elapsed between sample collection and fluorescence analysis which excludes any sample which was analysed for fluorescence more than 72 hours after sample collection.

4.4.1. Chemical Oxygen Demand (COD)

Table 4.2: Correlation coefficients (Spearman's Rho) for fluorescence parameters T_1, T_2, C and A measured by the author and Chemical Oxygen Demand (COD) as measured by the Environment Agency in samples mainly collected in South West England between March 2005 and February 2006. Population is analysed as all samples with surface water and effluent subsets.

	Peak T_1 275/340 nm	Peak T_2 225-237 /340-381nm	Peak C 300-370 /400-500nm	Peak A 237-260 /400-500nm	SMF-2 Output (T_1)
All Samples	0.848	0.790	0.851	0.825	0.888
Surface water	0.690	0.612	0.905	0.888	0.855
Effluent	0.697	0.723	0.688	0.815	0.767
Excluding >72 hrs					
All Samples	0.839	0.796	0.833	0.800	0.871
Surface water	0.661	0.617	0.899	0.881	0.847
Effluent	0.621	0.659	0.643	0.769	0.714

In total COD values were reported for 50 sites. Of these 36 were surface waters and 14 effluents (no time constraint). When samples analysed more than 72 hours after collecting were excluded 41 samples remained of which 28 were surface waters and 13 effluents.

In Chapter 3 the relationship between fluorescence and BOD_5 determined for all samples was stronger than for the individual sample groups (surface waters and effluents). However, the pattern is not true for COD. COD generally demonstrates only moderate correlation with any fluorescence peak in effluents. Surface waters demonstrate stronger correlations between the humic-like peaks and COD than the full set of samples (which are influenced

by the poorer relationship of the effluents). Furthermore correlations between humic-like fluorescence and COD are better than the correlations between $T_1$and T_2 fluorescence and COD in surface waters.

The strong relationship between peaks C, A and COD indicate that humic-like material in surface waters is more readily oxidised by the COD process than effluent humics. This suggests that surface water humic material is not as readily biodegraded as the humic material in effluents (reflected by the weaker BOD_5 relationship), but is more susceptible to chemical oxidation. This may be due to the degree of in-stream processing, the age and degree of humification of surface water humic materials. It is, however, contrary to the findings of Lee and Ahn (2004) and Reynolds, (2002) who found much stronger relationships between COD and tryptophan-like fluorescence than those reported here.

4.4.2. Total Organic Carbon (TOC)

Table 4.3: Correlation coefficients (Spearman's Rho) for fluorescence parameters T_1, T_2, C and A measured by the author and Total Organic Carbon (TOC) as measured by the Environment Agency in samples mainly collected in South West England between March 2005 and February 2006. Population is analysed as all samples with surface water and effluent subsets.

	Peak T_1 275/340nm	**Peak T_2 225-237 /340-381nm**	**Peak C 300-370 /400-500nm**	**Peak A 237-260 /400-500nm**	**SMF-2 Output (T_1)**
All Samples	0.826	0.786	0.863	0.826	0.833
Surface water	0.573	0.526	0.704	0.709	0.620
Effluent	0.764	0.577	0.719	0.604	0.728
Excluding >72 hrs					
All Samples	0.833	0.792	0.849	0.813	0.830
Surface water	0.527	0.478	0.623	0.624	0.594
Effluent	0.722	0.523	0.673	0.559	0.678

In total TOC results were reported for 527 sites. Of those 297 were surface waters and 230 effluents (no time constraint). When samples analysed more than 72 hours after collecting were excluded 416 samples remained of which 222 were surface waters and 194 effluents.

TOC shows the strongest correlation with peak C in line with the work of Baker, (2002a) and Clark et al., (2002). Although correlations suggest that T_1 fluorescence may be a good indicator of TOC, this work also supports the use of peak C fluorescence as an indicator of organic carbon concentration (Ferrari et al, 1996; Bieroza et al., 2009) as it demonstrates the strongest

correlation with TOC for the whole dataset which includes a broad range of sample types and sources.

4.4.3. Ammonia

Table 4.4: Correlation coefficients (Spearman's Rho) for fluorescence parameters T_1, T_2, C and A measured by the author and Ammonia as measured by the Environment Agency in samples mainly collected in South West England between March 2005 and February 2006. Population is analysed as all samples with surface water and effluent subsets.

	Peak T_1 275/340nm	**Peak T_2 225-237 /340-381nm**	**Peak C 300-370 /400-500nm**	**Peak A 237-260 /400-500nm**	**SMF-2 Output (T_1)**
All Samples	0.829	0.835	0.731	0.665	0.811
Surface water	0.473	0.397	0.371	0.346	0.442
Effluent	0.529	0.585	0.294	0.437	0.451
Excluding >72 hrs					
All Samples	0.829	0.832	0.761	0.724	0.806
Surface water	0.428	0.352	0.321	0.297	0.378
Effluent	0.564	0.585	0.282	0.441	0.450

In total ammonia results were reported for 323 sites. Of those 160 were surface waters and 163 effluents (no time constraint). When samples analysed more than 72 hours after collecting were excluded 267 samples remained of which 131 were surface waters and 136 effluents.

Ammonia correlates well with the T_1 and T_2 fluorescence regions in the total sample group, and also demonstrates a moderate relationship with peaks C

and A, however, in all cases the relationships fail when samples are split into surface waters and effluents.

4.4.4. Nitrate (NO_3^-)

Table 4.5: Correlation coefficients (Spearman's Rho) for fluorescence parameters T_1, T_2, C and A measured by the author and Chemical Oxygen Demand (COD) as measured by the Environment Agency in samples mainly collected in South West England between March 2005 and February 2006. Population is analysed as all samples with surface water and effluent subsets.

	Peak T_1 275/340nm	**Peak T_2 225-237 /340-381nm**	**Peak C 300-370 /400-500nm**	**Peak A 237-260 /400-500nm**	**SMF-2 Output (T_1)**
All Samples	-	-	-	-	-
Surface water	0.184	0.058	-0.081	-0.173	0.137
Effluent	-	-	-	-	-
Excluding >72 hrs					
All Samples	-	-	-	-	-
Surface water	0.060	-0.038	-0.174	-0.278	0.014
Effluent	-	-	-	-	-

In total nitrate results were reported for 281 surface water sites (no time constraint). When samples analysed more than 72 hours after collecting were excluded 225 surface waters remained.

Nitrate has no relationship with any fluorescence parameter. Nitrate may be unstable in stored samples, be bound in non-fluorescing molecules, or occur as discrete molecules with no fluorescent character.

4.4.5. Orthophosphate as P

Table 4.2: Correlation coefficients (Spearman's Rho) for fluorescence parameters T_1, T_2, C and A measured by the author and Chemical Oxygen Demand (COD) as measured by the Environment Agency in samples mainly collected in South West England between March 2005 and February 2006. Population is analysed as all samples with surface water and effluent subsets.

	Peak T_1 275/340nm	**Peak T_2 225-237 /340-381nm**	**Peak C 300-370 /400-500nm**	**Peak A 237-260 /400-500nm**	**SMF-2 Output (T_1)**
All Samples	-	-	-	-	-
Surface water	0.694	0.648	0.478	0.428	0.557
Effluent	-	-	-	-	-
Excluding >72 hrs					
All Samples	-	-	-	-	-
Surface water	0.682	0.622	0.499	0.439	0.538
Effluent	-	-	-	-	-

In total orthophosphate results were reported for 185 surface water sites (no time constraint). When samples analysed more than 72 hours after collecting were excluded 142 surface waters remained.

Orthophosphate, a nutrient which enhances microbial activity, correlates well with T_1 and T_2 which are believed to be microbial substrates or products (Cammack et al., 2004) indicating that orthophosphate is a part of the microbial metabolism production of tryptophan-like material.

4.5. The Importance of Time between Sample Collection and Analysis

The correlations between fluorescence and COD, TOC, Ammonia and orthophosphate are essentially unchanged when samples analysed more than 72 hours after collection are excluded. Nitrate demonstrates no relationship when these samples are included or excluded.

4.6. Discussion

The strength of the correlation between fluorophores and water quality parameters is heavily influenced by the type of sample. Water quality parameters which are indicators of poor water quality and pollution events e.g. COD and ammonia correlate more strongly with fluorescence in effluents than surface waters and most strongly with tryptophan-like fluorescence.

COD correlates better with T_1 and T_2 in effluents and with peaks C and A in surface waters. The surface water humic-like fluorescence/ COD correlation is substantially stronger than the effluent tryptophan-like fluorescence/ COD correlation. A large proportion of effluent organic matter tends to be younger, more labile and available for biodegradation. The storage and transport of these samples may have influenced these relationships. Fluorescence and COD analysis were not undertaken in the same location or necessarily at the same. If there was a time lag between COD analysis and fluorescence analysis during which the more labile (peak T) material was metabolised by the microbial community, the sample which was analysed for fluorescence would not be identical to that analysed for COD affecting the strength of the

relationship observed. The correlation of COD with less bioavailable humic-like fluorescent material (which is less likely to have been metabolised by the microbial community during sample transport and storage) is stronger.

This may explain why correlations in this work are different to those cited in the literature review (Lee and Ahn, 2004; Reynolds, 2002) in which better relationships are observed between COD and tryptophan-like material than humic-like material. However, it may also simply reflect that surface water organic matter predominantly comprises degraded terrestrial organics (humic-like fluorescence peaks) which, this suggests, require chemical attack to achieve total oxidation. It may also be a product of the fluorescence analysis, in which peak identification is not always obvious. Normal, unpolluted surface waters fluoresce weakly in the T_1/ T_2 regions but more strongly in the peak C and A regions, while effluents fluoresce strongly in all regions with peaks A and T_2 often being the most intense regions. The strength of the correlation with COD may be related to the quality of the fluorescence data in those regions that demonstrate the greatest fluorescence intensity.

It would be expected that TOC should demonstrate good correlation with older humic-like material in surface waters as in normal surface water this constitutes the vast majority of the organic carbon load. Thus we observe good correlations between TOC and peaks C and A, with peak C being commonly used as an indicator of organic carbon concentration. However, T_1 demonstrates an equally strong relationship with TOC in all samples and in effluents. In effluents peaks T_1 and C correlate equally well with TOC. In all

cases the correlation between T_2 and TOC is the weakest. As in previous chapters this is an example of T_1 and T_2 behaving in different ways to the same conditions.

The strength of the relationship between T_1 fluorescence and TOC may be an anomaly, influenced by the poorly defined peak shape often exhibited by the T_1 and C peaks and the tendency of the peaks to overlap, affecting the identification of peak position and fluorescence intensity. This is particularly true in the case of surface waters in which T_1 fluorescence intensity may be very low. Figure 4.1 illustrates the impact on the T_1 / TOC relationship of correcting T_1 fluorescence intensity for different proportions of peak C intensity to account for mutual interference. No improvement in the T_1 / TOC relationship is observed (up to a correction of -30% C intensity) indicating that masking of the T_1 peak by the neighbouring C peak is probably not a factor in the T_1/ TOC relationship. An improvement after correction for C might suggests that peak C was exerting an influence which was then removed by the correction.

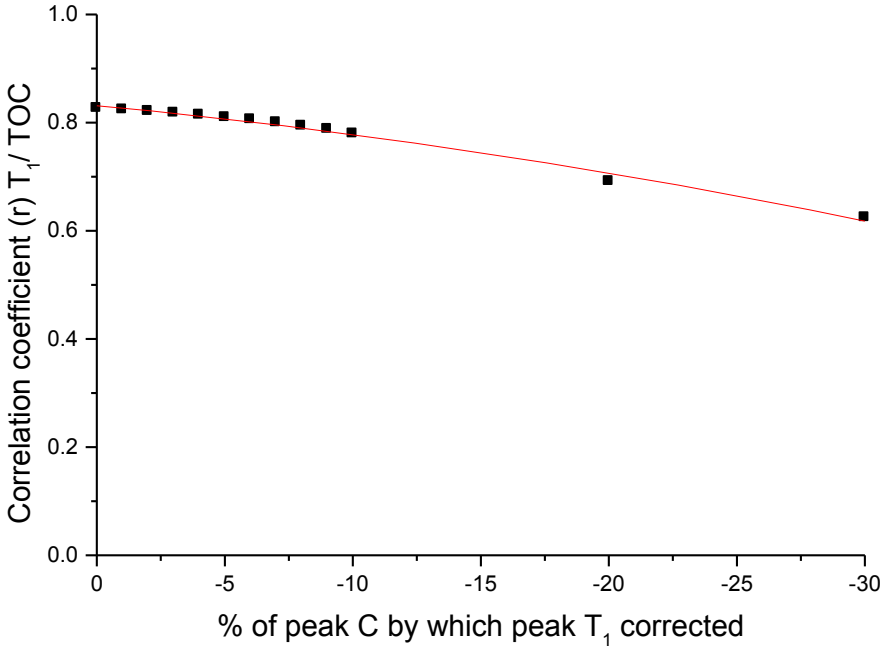


Figure 4.1: Plot of the Spearman's Rho (r) correlation coefficient determined between peak T_1 fluorescence intensity and TOC against percentage (%) of measured peak C fluorescence intensity (measured on the same EEM as T_1fluorescence intensity) which was subtracted from T_1 fluorescence intensity to account for the influence of peak C on peak T_1. Peak C is seen to have little influence on peak T_1 as indicated by the small change in correlation as T_1 is corrected, even after correction by up to 30% peak C fluorescence.

Peaks T_2 and A do not have the same degree of mutual association as they are generally found to exhibit more pronounced peaks in characteristic areas, and so are more easy to segregate into specific fluorophores.

As seen in the case of BOD_5 separating samples into individual types weakens the relationship between TOC and fluorescence but the patterns are less clear than were found for the BOD_5/ fluorescence relationship. The strength of the relationship between fluorescence and TOC is similar for

peaks T_2 and C and is similar in both effluents and surface waters. Peak T_1 correlates well with TOC in effluents and peak A is more strongly correlated with TOC in surface waters. This suggests that the contribution of labile (T_1 rich) fluorescent organic matter is greater in effluents than surface waters in which stable, degraded, terrestrial humic material make the greater contribution.

The remaining water quality parameters considered were measured in surface waters only. Ammonia demonstrates moderate correlations with peak T fluorescence while peaks C and A generally demonstrate similar moderate correlations. A correlation between T_1/ T_2 and ammonia in effluents is expected as ammonia is commonly associated with sewage discharges, for which T_1 and T_2 may be used as an indicator. The strength of the relationship between the tryptophan-like peaks and ammonia is contrary to the work of Baker et al., (2003; 2004), who identified strong relationships (0.95-0.98) between T_2 and ammonia, but only in the case of identifiable pollution events with high ammonia concentrations. In “normal” solutions e.g. river water there was no clear relationship (r=0.08). In general, the relationships in this work are stronger than those found under “normal” conditions in the work of Baker, (2003; 2004). Furthermore the relationship between tryptophan- like fluorescence and ammonia in effluents is less significant than the relationships Baker, (2003; 2004) found in the aftermath of pollution incidents. This is a result of the treatment received by waste waters to remove ammonia (to meet regulated nutrient standards) prior to discharge to rivers as effluent.

Orthophosphate has a moderate relationship with tryptophan-like fluorescence and is a food source to the microbial community which then excrete labile organic matter rich in tryptophan-like fluorescence. There is also a degree of association between orthophosphate and humic-like fluorescence (peaks C and A) which may indicate that the some of the orthophosphate present has a terrestrial source. Orthophosphate, however, does not demonstrate a strong correlation with organic carbon in these samples (r=0.309) suggesting that the majority of the orthophosphate present is not bound within organic molecules.

Nitrate demonstrates no relationship with any of the fluorescent moieties studied in this work. Correlations suggest that nitrate in these waters is not associated with organic carbon (r=0.344 organic carbon or humic-like fluorophores C and A, r=-0.049 and -0.052 respectively). This is a result of the character and structure of the humic acids present as these factors influence the potential for adsorption and uptake of nitrate (Klučáková, 2009).

As discussed in section 4.3.3 excluding data for samples which were analysed more than 72 hours after sample collection does not significantly alter the relationships observed. This suggests that any changes to fluorescent material during transport and storage which may alter the relationship with water quality parameters analysed elsewhere mainly occur within the first 72 hours after sample collection. After this point there is little influential change in the samples.

The reason for the reduction in the strength of relationships observed when the sample group is split into sample types is likely to be a result of the heterogeneous nature of natural waters. As stated in Chapter 3 each sample is from a site which is subject to specific environmental, seasonal and source influences all of which influence the chemistry of the water and the fluorescence of the organic matter present.

4.7. Summary

1. T_1 and T_2 correlate strongly with measures of poor water quality supporting the findings of Chapter 3 that they may be used as indicators of such and are related to nutrient induced microbial activity.

2. Humic-like fluorescence demonstrates good correlations with COD and TOC indicating that they are more recaltriant than the tryptophan-like moieties and contribute a greater proportion of the total organic carbon load. Peak A and C may indicate TOC concentration in surface waters, but should be used with caution in effluents in which peak T_1 demonstrates similar strength of correlation, indicating a similar contribution to the total organic carbon load.

3. After the first 72 hours the time lapse between sample collection and analysis is not relevant in these samples, provided samples are refrigerated. However, it is possible that for COD analysis samples should be analysed for fluorescence immediately as metabolism of the

labile protein-like material may occur during transport if storage conditions are not rigorously managed.

4. T_1 and T_2 fluorescence demonstrates sufficient correlation with ammonia that fluorescence spectroscopy has the potential to be used as a field indicator of this water quality measurement in natural and waste waters, although it may be necessary to develop site specific relationships in order for the technique to be robust.

5. This is not a suitable tool for indicating nitrate concentrations in these waters as the nitrate present is not directly associated with organic carbon. As nitrate binding with humic matter is influenced by the character of the humic material it is likely that, in these waters, little nitrate is bound but is present as independent or inorganic species. It would also not be recommended as an indicator of orthophosphate concentration as the relationships are not sufficiently robust and it is likely that only a proportion of the orthophosphate in water is associated with organic carbon, the rest may occur as independent or inorganic species demonstrating no fluorescence.

5. CHANGES IN FLUORESCENCE UNDER DIFFERENT CONDITIONS OF LIGHT AND TEMPERATURE

Rationale

An investigation into changes in organic matter fluorescence in water with time, under different storage conditions with a view to informing the debate about sample storage prior to analysis, transformations of organic matter in the aquatic environment and investigating possible sources and dynamics of individual fluorophores.

5.1. Introduction

In this chapter fluorescence excitation emission matrices (EEMs), microbiological cell counts and water chemistry parameters are interrogated to determine the effect of storage on the fluorescence of river water samples under a range of different environmental conditions. Section 1.4 of the main literature review details a number of authors who have investigated the effect of environmental conditions upon aquatic organic matter, particularly the effect of light exposure. Light exposure and photodegradation of organic matter affect the rate at which carbon is cycled from terrestrial plant sources, through the hydrosphere, to atmospheric carbon. Light also influences the behaviour of aquatic microbial communities affecting respiration, the process by which organic carbon from plant and algal sources is transformed to CO_2.

Previous published work has taken the form of storage stability trials, in-situ character measurements and short term laboratory based assessments of the effect of natural or artificial irradiance upon the character of the organic matter present. Changes in organic matter character predominantly concentrating on dissolved organic matter (DOM) have included measurement of changes in degree of humification; chemical structure; molecular size and weight; ratios of organic and inorganic carbon; photoaggregation; lability and bioavailability; absorbance properties and fluorescence properties (both fluorescence intensity and peak position) although fluorescence based studies are not common.

In this work four storage conditions were selected: an environmental proxy (11°C in light/ dark cycles, 11°C in the dark as a control) with 11°C adopted for this purpose as a maximum average environmental proxy for the Birmingham area (The Met Office); 4°C in the dark is the storage condition in work published by the fluorescence community; 20°C in the dark replicates the BOD_5 test conditions.

Tryptophan-like fluorescence is of particular interest as this is considered to be a direct measurement of biological activity, being a product of the microbiological community (Elliott et al., 2006; Cammack et al., 2004) and is also reflective of the more biodegradable fractions of organic matter present (Hudson et al., 2008). For this reason it is an important indicator of sample stability. It was anticipated that, as a microbial product or substrate, the tryptophan-like fluorescence intensity would change in line with changes in

cell count and that change would be greatest in the samples stored at 20°C in the dark, as this is the condition under which bacterial communities thrive in the BOD_5 test.

This work proposes to investigate the effect of light exposure upon river organic matter in samples removed from the environment under a range of controlled conditions. It will determine likely changes in organic matter composition and structure under these conditions by identifying changes in fluorescence character. These issues are relevant to sample storage and stability and may indicate potential mechanisms of fluorescent organic matter change in the wider environment. Results are presented for 3 size fractions so that an assessment may be made as to the relative photochemical and biological stability organic matter in total samples, dissolved and colloidal and dissolved only fractions.

5.2. Literature Review

Over time and with exposure to light DOC levels decrease (Corin et al., 1996) while DIC increases, indicating mineralisation of the organic carbon either through heterotrophic use or direct photomineralisation. The origin of DOC is thought to impact upon the degree and type of mineralisation that occurs. Terrestrial humics have been found to be more readily mineralised than algal humics (Obernosterer and Benner, 2004). Algal DOC is more likely to be mineralised by microbial activity and terrestrial DOM by photomineralisation processes with light enhancing the heterotrophic use of refractory DOC but

having no effect on the uptake of labile DOC (Anesio et al., 2000; Amado et al., 2006).

Within the humic materials group fulvic acids are more susceptible to photo- and biomineralisation than humic acids (Kulovaara, 1996; Schmitt-Kopplin et al., 1998) due to their higher phenol and lignin content, while humic acids are rich in less reactive lipids (Schmitt-Kopplin et al., 1998; Lepane et al., 2003). It is suggested that aromatic compounds are more photoreactive (Gonsoir et al., 2009) but that aliphatic materials are more rapidly photomineralised (Rodríguez-Zúñiga et al., 2008) and that molecules become less aromatic, and more aliphatic (Kulovaara, 1996; Osburn et al., 2001; Carvalho et al., 2008), with a reduction in carbohydrate content (Engelhaupt et al., 2003), an increase in carboxyl and carbonyl compounds (Gao and Zepp 1998; Engelhaupt et al., 2003), more hydrophobic and less hydrophilic properties (Cleveland et al., 2004) and generally reduced DOC heterogeneity (Lou and Xie, 2006).

Within an overall decrease in DOC a temporary increase in DOC is observed in the early part of the dark cycle in samples which are stored in light/ dark cycles rather than permanent light conditions (Hama et al., 2004). This is due to leakage of cell contents from the microfauna present (Hama et al., 2004) or production and excretion of DOM (Hertkorn et al., 2002; Urban-Rich et al., 2006) some of which is fluorescent (Urban-Rich et al., 2006). This is supported by the work of Moran et al. (2000) and Winter et al. (2007) while

Elliott et al. (2006) observe an increase in peak T fluorescence over time directly related to cell count in Pseudomonas aeruginosa.

Molecular size and weight is found to decrease with time and light exposure (Lou and Xie, 2006) in line with mineralisation of DOC to DIC (Gao and Zepp, 1998; Fukushima et al., 2001; Lepane et al., 2003). Lower molecular weight photo-products are proposed to be more bioavailable than high molecular weight materials, however, there is some inconsistency in data regarding whether DOC becomes more bioavailable and labile with photodegradation, much of which must come back to the question of the origin of the DOC (Judd et al., 2006; Abboudi et al., 2008; Vione et al., 2009). Lability is commonly measured by an increase in bacterial respiration or growth. An increase in respiration after irradiation of DOC is observed by Engelhaupt et al., (2003) and by Benner and Ziegler, (1999) in their review of the response of DOC to irradiation. However, other works including that of Amon and Benner, (1996), Obernosterer and Benner, (2004) and Stepanauskas et al., (2005) show a decrease in lability with time and light exposure sometimes with no associated DOC loss (Stepanauskas et al., 2005) while Kaiser and Sulzberger (2004) illustrate a decrease in labile low molecular weight products in river water after irradiation. Weigner and Seitzinger (2001) and Bertilsson et al. (2004) show no change in bioavailability in river and marine DOM respectively. Furthermore, photomineralisation of DOM is thought to cause changes in bacterial community composition (Judd et al., 2006; Pérez and Sommaruga, 2007) and structure (Abboudi et al., 2008). Komada et al. (2002) suggest that

changes in chemical composition and DOC content all relate to a move from reactive to stable forms of carbon with age.

Some studies investigate the effect of light upon humic substances which have either been extracted specifically for the purpose of the study (Kalbitz et al., 2003) or IHSS standards (Fu et al., 2006). While these are interesting as inter-technique comparisons they have limited use in determining the processes influencing environmental DOM. The extraction processes of drying, acidification and fractionation are all likely to have an impact on the physical properties, structure and chemistry of DOM and so any observed changes are unlikely to be representative of DOM in the natural environment. Standard substances are found to react differently to irradiation than natural DOM in direct comparison (Fu et al., 2006).

Optical properties are also clearly affected by irradiation. Again, it appears that the format of the experiment and the source of the organic material strongly influence the fluorescence character and behaviour of the DOM. Fluorescence of humic-like material is seen to decrease with storage and irradiation suggesting photomineralisation to non-fluorescent products (Patel-Sorrentino et al., 2004) with peak C (Skoog et al., 1996; Bertilsson et al., 2004) intensity declining to a greater extent than humic-like peak A (Moran et al., 2000; Lepane et al., 2003). Tryptophan-like fluorescence peak T is seen to either decrease or increase in fluorescence intensity (Moran et al., 2000; Winter et al., 2007), depending upon the source, “stability” and age of the sample. However, humic-like fluorescence intensity may also increase with

time (Skoog et al., 1996; Fukushima et al., 2001; Uyguner and Bekbolet, 2005) which would suggest either molecular change to expose more fluorophores or production of more fluorescent compounds which may be observed in the peak evolution identified in a humic acid standard by Fu et al. (2006) in which a peak at $\lambda_{ex/em}$ 275/500nm is seen to resolve into two peaks at $\lambda_{ex/em}$ 245/450nm and 310/450nm (peaks A and C) after irradiation. A blue-shift in peak position (excitation/ emission wavelength maxima) from higher to lower wavelength may also be seen after irradiation (Fu et al., 2006; Rodríguez-Zúñiga et al., 2008) which would be in line with a decrease in molecular size, but this change is not always evident (Fukushima et al., 2001; Saadi et al., 2006).

Finally, this work also presents an analysis of the effect of pH upon DOM fluorescence as increasing pH was identified in samples stored at 11°C in light/ dark cycle conditions, with the actual effect probably amplified by the small sample volumes used in the study. Increases in pH in incubated samples have previously been observed (up to 2 pH units) by Schmitt-Kopplin et al. (1998) and Engelhaupt et al. (2003) although the direct influence of this on the change in DOM character observed is not reported. Rather, this is suggested to be a result of the formation of acidic substances (Gao and Zepp, 1998) and related to the growth of phytoplankton (Engelhaupt et al., 2003). Fluorescence is not an analytical technique used in these works. It is well known that pH affects fluorescence intensity (see section 1.4.1) and this is investigated through manipulation of pH in a number of studies including

those by Vodacek and Philpot, (1987); Patel-Sorrentino et al., (2002); Baker et al., (2007) and Spencer et al., (2007).

The result of changing pH on fluorescence properties of water are generally common across all studies whether pH is changed by +/- 2 pH units from original (Baker et al., 2007) or across a range of pH from 2-11 (Patel-Sorrentino et al., 2002) although individual fluorophores react in different ways. Tryptophan-like fluorescence intensity is little changed by changing pH (Baker et al., 2007; Spencer et al., 2007) although Sheng and Yu, (2006) observe an increase in fluorescence intensity of the T_2 peak in sludges. Humic-like substance fluorescence intensities are found to be more reactive, increasing with increasing pH (2-10) (Mobed et al., 1996; Pullin and Cabaniss, 1997; Patel-Sorrentino et al., 2002; Spencer et al., 2007) before dropping off at pH 11 (Patel-Sorrentino et al., 2002; Sheng and Yu, 2006; Spencer et al., 2007). Peak A is always more sensitive to pH change than peak C, showing an enhanced increase in fluorescence intensity (Patel-Sorrentino et al., 2002). In addition increasing pH often leads to a red-shift (shift to longer wavelength) of the emission maxima of humic substances (Pullin and Cabaniss, 1997; Sheng and Yu, 2006; Spencer et al., 2007). Humic substances responses to changing pH are likely to be a result of molecular shrinkage (Avena et al., 1999), conformational change and/ or a change in functional acid groups (Mobed et al., 1996).

Water sample storage protocols, for fluorescence and water chemistry analysis, aim to minimise the effect of light exposure and microbial activity.

Marine samples are often filtered to between 0.7μm and 0.22μm immediately after collection and are stored in the dark or in amber bottles in cool conditions. Samples may be re-filtered immediately prior to analysis. Samples from more distal sites may be frozen for transport. The impact of freezing is not covered in this paper but is addressed in Hudson et al., 2009. River water samples are also often filtered to between 0.7μm and 0.2μm (0.45μm is very common as this is a nominal cut off for the removal of surface water particulate material); although a total lack of filtration is more common in river water samples than marine samples. It is also usual for river water samples to be refrigerated under dark conditions and it is uncommon for river water samples to be frozen for storage. Furthermore samples of both marine and river water have occasionally been fixed by the addition of sodium azide (Ferrari et al., 1996; Patel-Sorrentino et al., 2004) or by acidification to pH2 by the addition of HCl (Kalbitz et al., 2000). The impact of chemical preservation of samples in not addressed in this work. It is uncommon for an assessment of sample fluorescence change during storage to be reported.

Samples recovered for water chemistry analysis are also stored to minimise sample change. For TOC (DOC) analysis samples are refrigerated unfiltered (filtered) in the dark. If analysed in excess of 2 hours after collection pH is adjusted to <2 using HCl or H_2SO_4. Samples for conductivity measurement should be analysed within 24 hours of collection or filtered to 0.45μm and stored at 4°C while samples for pH measurement should be analysed immediately or stored in bottles filled completely and sealed to the atmosphere (Columbia Analytical Services Ltd.).

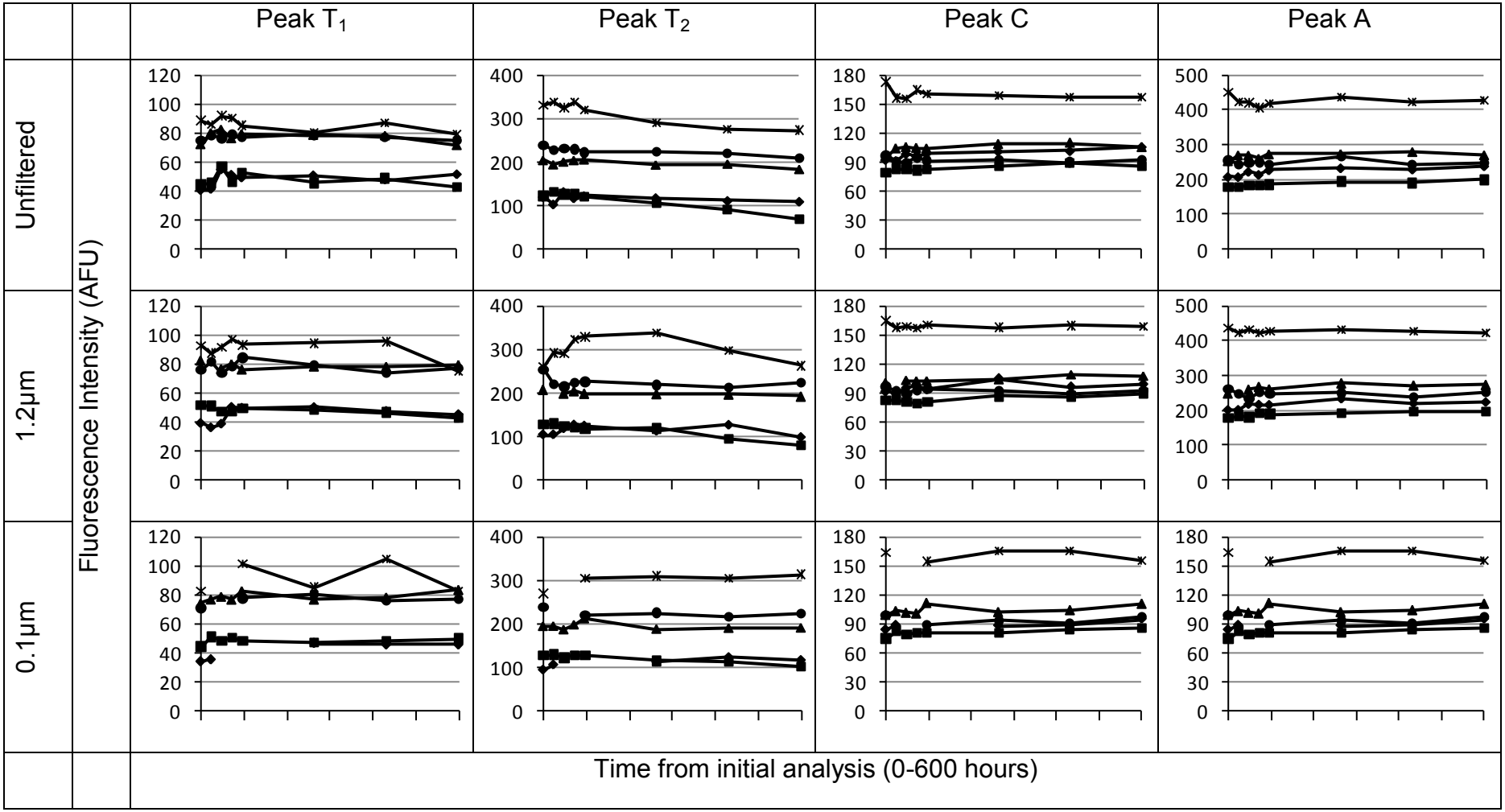
It is generally accepted that filtration and storage of samples in the dark in cool conditions is the best approach for minimising sample fluorescence change over time. To test this assertion the effects of light exposure and temperature upon freshwater organic matter fluorescence and water chemistry, in samples removed from the environment, are presented in both the short and long terms for samples stored in light and dark conditions, under a range of temperatures and in various size fractions. An assessment will be made of the efficacy of filtration, refrigeration and light minimisation in sample storage with a suggestion of best practice procedures for the storage of samples for fluorescence analysis.

This chapter presents changes in fluorescence properties, water chemistry and microbial abundance in river waters in relation to environmental conditions, with a view to investigating environmental processing of aquatic organic matter and its implications for sample storage protocols.

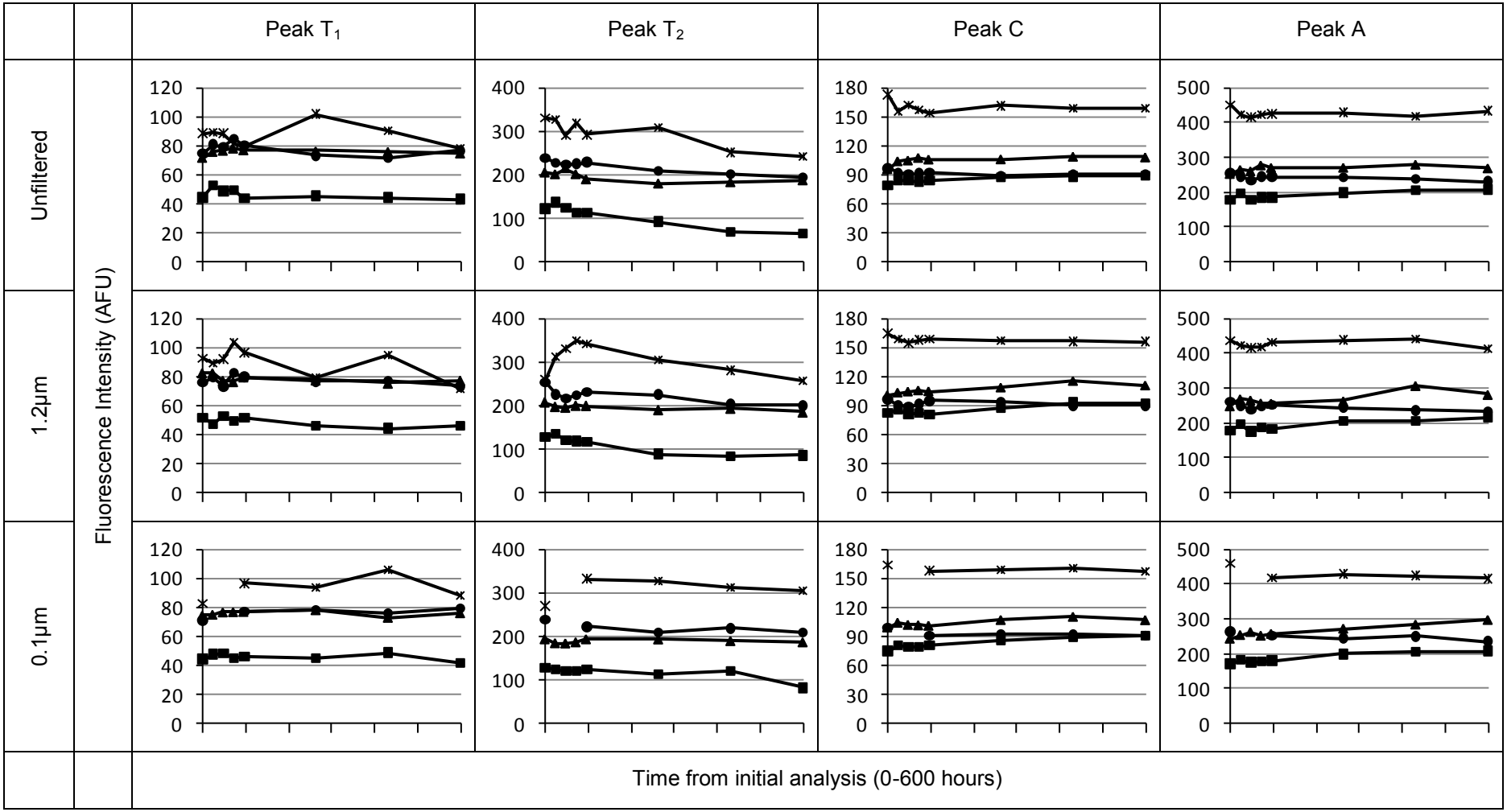
5.3. Results

The data presented in this chapter comprise mean values from triplicate fluorescence analyses.

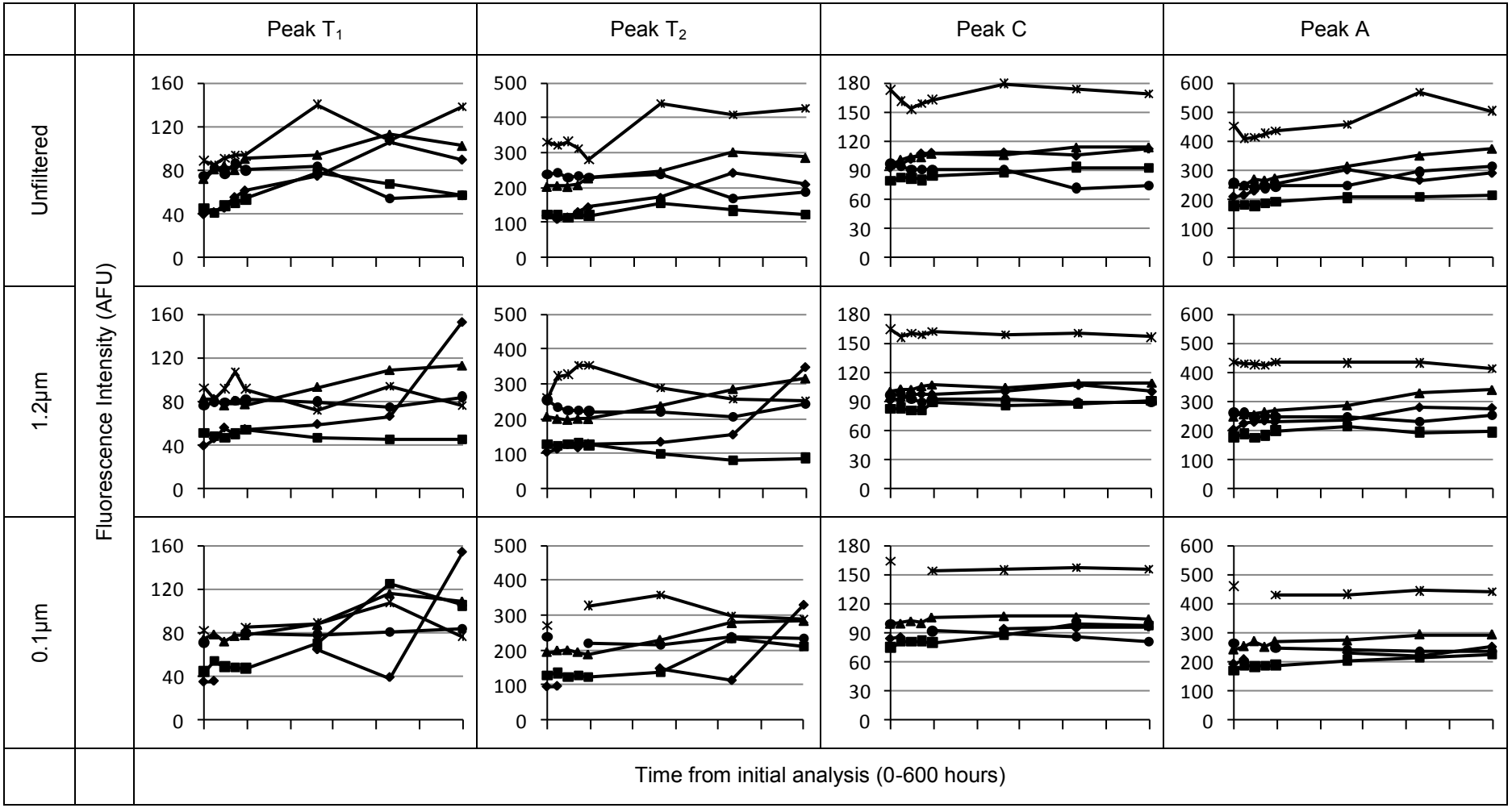
5.1a. Changes in mean fluorescence intensity in all samples (River Dove (diamond), Repton Brook (square), River Rea (triangle), River Tame (circle), Bourne Brook (cross)) at 0, 24, 48, 72, 96, 264, 432 and 600 hours after sample collection and after storage at 4°C in the dark.



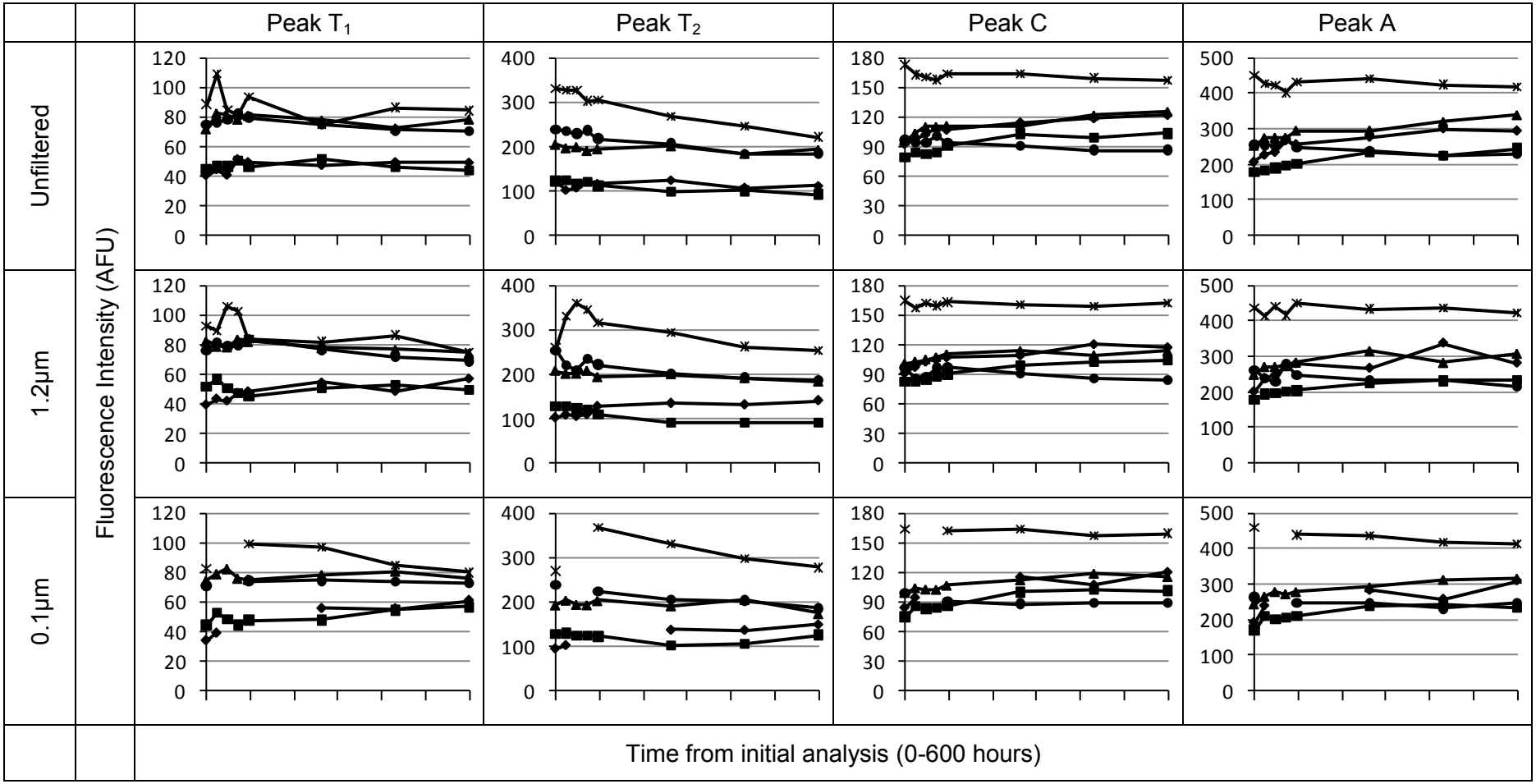
5.1b. Changes in mean fluorescence intensity in all samples (River Dove (diamond), Repton Brook (square), River Rea (triangle), River Tame (circle), Bourne Brook (cross)) at 0, 24, 48, 72, 96, 264, 432 and 600 hours after sample collection and after storage at 11°C in the dark.



5.1c. Changes in mean fluorescence intensity in all samples (River Dove (diamond), Repton Brook (square), River Rea (triangle), River Tame (circle), Bourne Brook (cross)) at 0, 24, 48, 72, 96, 264, 432 and 600 hours after sample collection. Storage at 11°C in light / dark cycles.



5.1 d. Changes in mean fluorescence intensity in all samples (River Dove (diamond), Repton Brook (square), River Rea (triangle), River Tame (circle), Bourne Brook (cross)) at 0, 24, 48, 72, 96, 264, 432 and 600 hours after sample collection and after storage at 20°C in the dark.



5.3.1. ANCOVA Analysis of Changes in Fluorescence and Water Chemistry with Light Exposure, Temperature and Filtration

Figures 5.1 a-d illustrate the changes observed in mean fluorescence intensity during the analytical period under the specified conditions. ANCOVA (ANalysis of COVAvariance) was undertaken upon the raw data to compare the changes and differences in fluorescence and water chemistry values for the means from the 6 samples over time under different storage conditions. The effects of light exposure and storage temperature were analysed in separate models in which filtration and light exposure or filtration and temperature were identified as covariant factors and the storage time as a fixed factor. Raw data used in the individual models (light exposure ad temperature) are presented in Appendices 3 and 4 respectively.

The main effects of storage period, exposure to light and degree of filtration when samples are stored at constant temperature are illustrated in Table 5.1.

Table 5.1: F-ratios for the main effects of fixed factor storage period and covariate factors light exposure and filtration upon the means of peak T_1, T_2, C, A fluorescence intensity and water chemistry parameters DO, pH, Conductivity, TOC for 6 samples (River Dove, Repton Brook, River Rea, River Tame, Bourne Brook, 18MΩ deionised water). Shaded cells indicate those parameters for which change with time is significantly influenced by light exposure and/ or filtration (p< .05).

Parameter	**Storage Period**	**Light**	**Fraction**
	F-ratio	*F*-ratio	*F*-ratio
T_1 intensity	10.15	16.52	7.41
T_2 intensity	1.86	3.92	8.49
C intensity	1.69	0.60	6.65
A intensity	3.73	0.52	22.92
DO	25.47	192.53	46.05
pH	13.13	132.43	11.02
Conductivity	0.64	17.97	0.77
TOC	25.95	56.34	49.12

Significant changes in mean are observed (for the cumulative data for 6 samples) in peaks T_1 and A, DO, pH and TOC in samples from these conditions during the storage period. Exposure to light/ dark cycles significantly influences the change in mean of T_1 and T_2 fluorescence intensity and all water chemistry parameters over the storage period. Significantly different mean values are identified for most analyses between the different filtered fractions. However, change in conductivity with time is significantly affected by exposure to light but the degree of mean change does not vary significantly between filtered fractions. The humic-like peaks C and A demonstrate significant differences in mean change between the filtered fractions but are not significantly influenced by exposure to light.

The impact of filtration is illustrated in Table 5.2 in which significant differences in means are identified between the different filtered fractions.

Table 5.2: t-test results for the differences in the means of peak T_1, T_2, C, A fluorescence intensity and water chemistry parameters DO, pH, Conductivity, TOC for 6 samples (River Dove, Repton Brook, River Rea, River Tame, Bourne Brook, 18MΩ deionised water) over time between different filtered fractions. Shaded cells indicate those parameters for which difference between mean values in different filtered fractions is significant (p< .05).

Parameter	**Unfiltered/ 1.2µm**	**1.2µm/ 0.1µm**
	t-test value	*t*-test value
T_1 intensity	3.13	3.65
T_2 intensity	3.67	4.81
C intensity	3.14	5.94
A intensity	4.10	6.05
DO	5.69	-2.43
pH	2.70	-4.88
Conductivity	0.51	-1.53
TOC	6.76	0.78

In samples stored at constant temperature under different conditions of light/ dark all parameters demonstrate significantly different means across the different filtered fractions except conductivity for which mean values are not significantly different.

The main effects of storage temperature and filtered fraction in samples stored in the dark are illustrated in Table 5.3.

Table 5.3: F-ratios for the main effects of fixed factor storage period and covariate factors temperature and filtration upon the means of peak T_1, T_2, C, A fluorescence intensity and water chemistry parameters DO, pH, Conductivity, TOC for 6 samples (River Dove, Repton Brook, River Rea, River Tame, Bourne Brook, 18MΩ deionised water). Shaded cells indicate those parameters for which change with time is significantly influenced by temperature and/ or filtration (p< .05).

Parameter	**Storage Period**	**Temperature**	**Fraction**
	F-ratio	*F*-ratio	*F*-ratio
T_1 intensity	5.15	0.01	3.23
T_2 intensity	6.14	0.49	3.17
C intensity	2.00	6.40	2.54
A intensity	2.13	8.02	2.96
DO	199.75	620.02	0.02
pH	47.91	376.96	322.80
Conductivity	3.17	0.10	8.76
TOC	9.90	1.30	30.34

All fluorescence and water chemistry parameters demonstrate significantly different means across the storage period. However, changes in the tryptophan-like peaks do not appear to be a function of storage temperature or filtration. Significant differences are observed in the mean fluorescence intensity of peaks C and A and also DO and pH between samples stored at 4°C, 11°C and 20°C. Significant differences are observed in the mean values of pH, Conductivity and TOC in different filtered fractions.

Table 5.4: t-test results for the differences in the means of peak T_1, T_2, C, A fluorescence intensity and water chemistry parameters DO, pH, Conductivity, TOC for 6 samples (River Dove, Repton Brook, River Rea, River Tame, Bourne Brook, 18MΩ deionised water) over time between different storage temperatures and filtered fractions. Shaded cells indicate those parameters for which difference between mean values in samples from different storage temperatures and filtered fractions is significant (p< .05).

Parameter	**4°C/ 11°C**	**11°C/ 20°C**	**Unfiltered/ 1.2µm**	**1.2µm/ 0.1µm**
	t-value	*t*-value	*t*-value	*t*-value
T_1 intensity	-0.17	3.29	2.78	7.17
T_2 intensity	0.49	3.14	2.53	6.57
C intensity	-2.54	-0.96	2.37	6.94
A intensity	-2.87	-0.72	2.37	7.07
DO	16.91	7.69	-1.11	2.61
pH	18.83	10.48	-17.99	-13.32
Conductivity	-0.38	0.68	2.20	-3.02
TOC	1.04	0.97	5.59	2.49

Table 5.4 illustrates variations in the means of the fluorescence and water chemistry analyses between the different storage temperatures and filtered fractions. The means of the fluorescence and water chemistry parameters are significantly different between the unfiltered and 1.2µm fractions and between the 1.2µm and 0.1µm fractions (although DO is anomalous in that there is no significant difference in means between unfiltered and 1.2µm filtered samples).

Significant differences are observed in the means of the humic like peaks between samples stored at 4°C and 11°C, although not between those stored at 11°C and 20°C while the tryptophan-like peaks demonstrate the opposite trend. The “direction” of the t-value (+ or –) suggests that mean humic-like fluorescence intensity is significantly lower in samples stored at 4°C than

those stored at 11°C indicating increased evolution of these peaks at higher temperatures (- values). Tryptophan-like intensity demonstrates different patters between the T_1 and T_2 peaks, but predominantly suggests that the mean intensity is significantly higher at 11°C than at 20°C (+ values). This suggests loss of this material with increasing temperatures. This may be an indication of tryptophan-like fluorescent material as a bacterial substrate and humic-like fluorescent material as a bacterial product.

Later sections in this chapter discuss these findings in more detail and incorporate data relating to growth of colony forming units, algae and changes in fluorophore peak positions.

Table 5.5a: Percentage change in mean (of triplicate analysis) observed fluorescence intensity between 0 hours and 24, 96 and 600 hours after sample collection in unfiltered, 1.2µm and 0.1µm filtered fractions of each sample stored at 4°C in the dark. Note unfiltered samples represented by "Unf"

Peak T_1

	River Dove			Repton Brook			River Rea			River Tame			Bourne Brook		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	+2	-8	+4	+2	0	+16	+12	nd	+4	+4	+8	nd	-3	-5	nd
96 hours	+22	+27	nd	+18	-3	+10	+11	-7	+12	+3	+12	+9	-4	+1	+22
600 hours	+28	+16	+32	-3	-17	+13	-1	-4	+13	0	+1	+9	-11	-9	0

Peak T_2

	River Dove			Repton Brook			River Rea			River Tame			Bourne Brook		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	-14	0	+13	+6	0	+1	-5	nd	0	-5	-13	nd	+2	+12	nd
96 hours	+2	+20	nd	-2	-8	-1	+1	-4	+9	-7	-11	-8	-3	+27	+13
600 hours	-9	-6	+24	-45	-38	-21	-11	-7	-2	-12	-12	-6	-17	+1	+16

Peak C

	River Dove			Repton Brook			River Rea			River Tame			Bourne Brook		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	-1	-2	+6	+5	+1	+11	+8	nd	+4	-6	-5	nd	-10	-4	nd
96 hours	+7	+2	nd	+4	-1	+8	+8	+2	+12	-5	-2	-10	-7	-3	-6
600 hours	+15	+8	+13	+8	+9	+15	+10	+7	+11	-4	-4	-2	-9	-3	-5

Peak A

	River Dove			Repton Brook			River Rea			River Tame			Bourne Brook		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	0	0	+1	0	+4	+15	+7	nd	+7	-4	-6	nd	-6	-3	nd
96 hours	+9	+8	nd	+5	+7	+7	+8	+4	+11	-4	-5	-9	-8	-2	-8
600 hours	+15	+12	+19	+12	+10	+14	+7	+9	+18	-2	-4	-5	-5	-3	-7

Table 5.5b: Percentage change in mean (of triplicate analysis) observed fluorescence intensity between 0 hours and 24, 96 and 600 hours after sample collection in unfiltered, 1.2µm and 0.1µm filtered fractions of each sample stored at 11°C in the dark. Note unfiltered samples represented by "Unf"

Peak T_1

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	nd	nd	nd	+19	-8	+8	+6	0	+1	+8	+4	nd	+1	-3	nd
96 hours	nd	nd	nd	-2	0	+5	+7	-3	+4	+7	+5	+9	-11	+5	+17
600 hours	nd	nd	nd	-3	-10	-6	+4	-6	+2	+2	-3	+11	-12	-22	+7

Peak T_2

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	nd	nd	nd	+11	+5	-4	-2	-5	-5	-5	-11	nd	-1	+20	nd
96 hours	nd	nd	nd	-9	-10	-2	-8	-4	0	-4	-8	-7	-12	+31	+23
600 hours	nd	nd	nd	-47	-34	-36	-9	-10	-4	-19	-21	-13	-27	-1	+13

Peak C

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	nd	nd	nd	+7	+4	+8	+9	+3	+5	-5	-6	nd	-10	-4	nd
96 hours	nd	nd	nd	+5	-2	+8	+10	+4	+2	-4	-1	-8	-11	-3	-4
600 hours	nd	nd	nd	+12	+12	+21	+13	+10	+7	-6	-7	-9	-8	-5	-4

Peak A

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	nd	nd	nd	+11	+11	+7	+5	+8	+4	-4	-4	nd	-6	-3	nd
96 hours	nd	nd	nd	+5	+3	+5	+7	+3	+6	-4	-3	-5	-6	-1	-9
600 hours	nd	nd	nd	+17	+22	+21	+6	+13	+22	-9	-11	-6	-4	-5	-10

Table 5.5c: Percentage change in mean (of triplicate analysis) observed fluorescence intensity between 0 hours and 24, 96 and 600 hours after sample collection in unfiltered, 1.2µm and 0.1µm filtered fractions of each sample stored at 11°C in light/ dark cycles. Note unfiltered samples represented by "Unf"

Peak T_1

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	+4	+17	+2	-9	-6	+21	+12	0	+6	+9	+6	nd	-4	-11	nd
96 hours	+53	+39	nd	+20	+5	+7	+25	-7	+5	+7	+8	+12	+5	-1	+3
600 hours	+122	+293	+346	+27	-11	+138	+42	+37	+47	-24	+11	+17	+56	-17	-7

Peak T_2

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	-9	+9	+2	-2	-5	+5	+1	-3	+3	+2	-8	nd	-3	+24	nd
96 hours	+20	+23	nd	-2	-3	-4	+12	-3	-4	-5	-13	-9	-16	+35	+21
600 hours	+57	+237	+248	+1	-32	+65	+40	+53	+46	-22	-4	-2	+30	-3	+7

Peak C

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	+3	0	+2	+3	+1	+8	+5	+3	0	-2	+1	nd	-7	-5	nd
96 hours	+17	+6	nd	+6	+8	+7	+12	+7	+7	-7	-4	-8	-6	-2	-7
600 hours	+21	+10	+15	+16	+10	+30	+19	+9	+5	-23	-7	-19	-2	-5	-5

Peak A

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	+3	+11	+8	+2	+6	+10	-3	+3	+4	-3	0	nd	-9	-1	nd
96 hours	+20	+13	nd	+9	+12	+10	+8	+7	+11	-4	-6	-6	-4	0	-7
600 hours	+39	+38	+30	+20	+10	+32	+48	+36	+20	+23	-3	-11	+12	-5	-5

Table 5.5d: Percentage change in mean (of triplicate analysis) observed fluorescence intensity between 0 hours and 24, 96 and 600 hours after sample collection in unfiltered, 1.2µm and 0.1µm filtered fractions of each sample stored at 20°C in the dark. Note unfiltered samples represented by "Unf"

Peak T_1

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	+10	+11	+14	+4	+11	+19	+15	-4	+6	+2	+8	nd	+23	-3	nd
96 hours	+24	+24	nd	+3	-12	+8	+13	0	+1	+6	+10	+4	+6	-10	+20
600 hours	+23	+46	+77	-1	-4	+28	+9	-10	+2	-7	-9	+2	-5	-19	-3

Peak T_2

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	-16	+6	+8	+2	-1	+2	-4	-3	+6	-1	-13	nd	-1	+27	nd
96 hours	-3	+23	nd	-9	-14	-4	-5	-7	+5	-9	-13	-6	-8	+21	+36
600 hours	-8	+35	+57	-25	-30	-2	-6	-11	-10	-23	-27	-23	-33	-2	+3

Peak C

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	+5	+7	+12	+6	+1	+15	+8	+2	+5	-3	-11	nd	-6	-5	nd
96 hours	+15	+18	nd	+14	+9	+14	+16	+10	+7	-2	0	-9	-5	-1	-1
600 hours	+32	+29	+43	+30	+27	+35	+31	+13	+16	-10	-12	-11	-9	-1	-3

Peak A

	River Dove			**Repton Brook**			**River Rea**			**River Tame**			**Bourne Brook**		
Fraction	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm	Unf	1.2µm	0.1µm
24 hours	+9	+19	+24	+3	+9	+23	+7	+9	+9	0	-8	nd	-5	-5	nd
96 hours	+23	+39	nd	+13	+15	+22	+17	+14	+14	-3	-5	-7	-4	+3	-5
600 hours	+41	+40	+60	+38	+32	+36	+34	+23	+30	-10	-18	-6	-7	-3	-10

Tables 5.5 a, b, c and d show the percentage change in fluorescence intensity in all samples after 24 hours, 96 hours and 600 hours (1 day, 5 days and 26 days) when stored under different conditions. All samples show a change in fluorescence intensity throughout the 600 hour analytical period. It is most common for the extent of change to be greater (in either direction +/-) after 600 hours.

On a day-to-day basis, after only 24 hours a maximum change in fluorescence intensity of up to +16% (peak T_1 in 0.1µm filtered samples) which may be an artefact of filtration – probably by cell lysis , or -14% (peak T_2 in unfiltered samples), is observed in samples stored at 4°C in the dark. This suggests that despite being the recommended best practice for short term sample preservation, fluorescence in samples stored at 4°C in the dark is still variable, particularly the more labile peaks T_1 and T_2 but the degree and direction of change is sample specific. Change is observed in almost all peaks under all conditions after only 24 hours, the degree of change being greatest in samples stored at 20°C in the dark and appears to be lowest in samples stored at 11°C in light/ dark cycles.

After 96 hours (representative of the BOD_5 test) the recorded change in intensity between 0 - 24 hours and 0 - 96 hours is very similar in samples stored at 4°C in the dark and 11°C in the dark suggesting that most change occurs in the first 24 hours. This is particularly true in the River Rea and River Tame samples. Over this period all peaks in samples stored at 11°C in light/

dark cycles demonstrate a greater degree of change than that observed in the 11°C dark control and in samples stored at 20°C in the dark.

By 600 hours the change in peaks C and A exceeds that of T_1 and T_2 in samples stored at 20° in the dark but in samples stored at 11°C in light/ dark cycles fluorescence change in the tryptophan-like peaks exceeds that of the humic-like peaks. A decrease in humic-like fluorescence intensity would be expected (Skoog et al., 1996; Bertilsson et al., 2004) with peak C intensity expected to decline to a greater extent than peak A (Moran et al., 2000; Lepane et al., 2003), however, peak C shows no significant difference between samples stored in light / dark cycles and those stored in the dark while peak A shows significant variation but only in the unfiltered fraction. Samples stored at 11°C in the dark demonstrate highly variable degrees and directions of change while samples stored at 4°C in the dark tend to demonstrate an increase in humic-like fluorescence of up to +15% in unfiltered samples but a range of responses in tryptophan-like fluorescence.

5.3.2. Growth of Colony Forming Units

Growth of bacterial colonies (Colony Forming Units (CFU)) is seen in all samples from all storage conditions and all filtered fractions. Highest growth levels are generally observed in samples stored at 20°C, in unfiltered fractions and on reduced nutrient media. Samples stored at 11°C in the dark generally demonstrate a higher CFU count by day 26 than samples stored at 11°C in light/ dark cycles. Samples stored at 4°C dark show the lowest overall growth

rate, with unfiltered samples again showing the greatest number of CFU by day 26. Samples filtered to 0.1µm do show an increase in CFU over the analysis period, although it is substantially lower in most cases than the other fractions, rarely exceeding 20,000 CFU/ml^{-1} over the 26 days. Most samples stored in the dark demonstrate restricted growth on yeast extract agar (YEA) and higher CFU counts on reduced nutrient agar (R2A), although samples stored in light/ dark cycles tend to demonstrate higher growth on yeast extract agar. The fraction likely to show the lowest level of bacterial growth is the 0.1µm fraction grown on YEA (nutrient rich) media suggesting that the microbial communities in the river waters analysed is more likely to thrive in a restricted nutrient environment. The number of CFUs recorded for each filtered fraction of each sample, stored under each environmental condition is reported in Table 5.6.

Table 5.6: Total number of colony forming units (CFU) recorded after 600 hours in nutrient rich (YEA) and nutrient poor (R2A) agar plates from unfiltered, 1.2µm and 0.1µm filtered fractions of each surface water stored at 4°C in the dark, 11°C in the dark, 11°C in light/ dark cycles and at 20°C in the dark. (Unf indicates unfiltered samples).

	Storage Temperature	**Total CFU/ml^{-1} on day 26, yeast extract agar and reduced nutrient (R2A) agar.**									
		River Dove		Repton Brook		River Rea		River Tame		Bourne Brook	
		YEA	R2A	YEA	R2A	YEA	R2A	YEA	R2A	YEA	R2A
Unf	4°C dark	42000	59000	5000	79000	21000	43000	150000	120000	49000	69000
	11°C dark	-	-	82000	110000	45000	111000	135000	254000	500000	178000
	11°C light/ dark	80000	200000	45000	5000	16000	6000	165000	13000	210000	170000
	20°C dark	127000	160000	86000	203000	85000	167000	208000	240000	1140000	1220000
1.2µm	4°C dark	16000	10000	30000	11000	14000	27000	33000	73000	42000	17000
	11°C dark	-	-	41000	32000	26000	89000	89000	285000	41000	28000
	11°C light/ dark	29000	266000	26000	3000	51000	2000	43000	15000	19000	4000
	20°C dark	114000	280000	51000	157000	18000	208000	910000	2280000	25000	14000
0.1µm	4°C dark	0	1000	1000	5000	3000	6000	6000	2000	0	4000
	11°C dark	-	-	3000	8000	0	17000	12000	1000	6000	15000
	11°C light/ dark	83000	2000	2000	0	11000	2000	7000	1000	5000	0
	20°C dark	11000	16000	3000	7000	10000	23000	179000	156000	2000	16000

5.3.3. Influence of Method Error on Fluorescence Intensity Change

All reported fluorescence data is a mean of values recorded in triplicate on the same portion of the same sample. Thus it should be possible to derive a measure of analytical uncertainty for the method by considering standard deviation from the mean to determine whether observed changes in fluorescence intensity are a result of change in fluorescence properties of the water or simply variation within the range of analytical uncertainty intrinsic to the method.

The standard deviation was calculated as a percentage of the mean fluorescence value for each filtered fraction from each environmental condition for all samples. The maximum standard deviation as percentage mean fluorescence intensity for each peak was recorded and is considered to be the maximum degree of fluorescence change (as a percentage) that may be attributed to analytical uncertainty. Thus, where percentage change observed is lower than the maximum value of standard deviation it is considered that this change may be attributed simply to method error. Where percentage change in intensity observed is greater than the maximum standard deviation it is assumed that fluorescence intensity change occurs within the sample as a result of chemical and or biological change. Table 5.7 illustrates the results of this analysis. Fluorescence intensity changes which exceed the range of analytical uncertainty are highlighted.

It can be seen that almost all changes in T_1 fluorescence intensity may be attributed to analytical uncertainty, except those stored at 11°C in light/ dark cycles. Peak T_2 changes in fluorescence intensity are less clearly attributable to method variation, although changes in samples stored at 4°C in the dark are generally within the bounds of analytical uncertainty. Changes in the fluorescence intensity of peaks A and C may also be attributed to method error in samples stored at 11°C in the dark. However, changes in samples stored at 11°C in light/ dark cycles and 20°C dark conditions clearly occur as a result of change in sample chemistry.

Table 5.7: Illustration of change in fluorescence intensity which may be attributable to analytical uncertainty. Analytical uncertainty in this instance has been classed as the maximum standard deviation from the mean of fluorescence intensity for each peak observed between the 5 surface water samples River Dove, Repton Brook, River Rea, River Tame and Bourne Brook.

		T1 % change	**Min s.d**	**Max s.d**	**T2 % change**	**Min s.d**	**Max s.d**	**C % change**	**Min s.d**	**Max s.d**	**A % change**	**Min s.d**	**Max s.d**
4°C Dark	Unf	+2	0.00	12.18	-19	0.00	10.18	+4	0.60	7.19	+5	0.23	10.37
	1.2µm	-4	0.63	18.00	-12	0.37	14.87	+3	0.58	4.61	+5	0.95	10.75
	0.1µm	+13	1.82	20.10	+2	0.64	18.43	+6	0.00	6.84	+8	0.36	13.25
11°C Dark	Unf	-2	1.08	27.08	-25	0.86	14.06	+3	0.89	7.19	+3	0.71	5.44
	1.2µm	-10	1.04	15.64	-17	1.08	13.47	+3	0.58	7.36	+5	0.62	8.78
	0.1µm	+4	1.05	20.10	-10	0.45	14.93	+4	0.00	4.54	+6	0.59	13.25
11°C L / D	Unf	+45	0.74	19.94	+25	0.00	11.60	+6	0.00	7.19	+29	0.33	16.82
	1.2µm	+61	1.16	18.46	+51	1.21	12.39	+30	0.46	7.97	+15	0.35	11.58
	0.1µm	+108	0.99	20.10	+73	0.32	18.43	+51	0.00	6.49	+13	0.35	13.25
20°C Dark	Unf	+4	0.65	20.68	-19	0.31	11.54	+15	0.42	7.19	+19	0.39	4.90
	1.2µm	+1	0.00	15.64	-7	0.80	16.37	+11	0.40	5.59	+15	0.48	6.74
	0.1µm	+21	1.09	20.10	+5	1.26	18.43	+16	0.00	5.48	+22	0.32	13.25

This should be considered through this chapter where fluorescence intensity changes will be reported and discussed with a view to investigating the effect of pH change, light and temperature on sample fluorescence and chemistry.

5.3.4. Modelling the Influence of pH Change on Fluorescence Intensity

Changes in pH after filtration were observed by Baker et al., (2007) with a decrease of 1pH unit after filtration to 1.2µm and up to 7 pH units after filtration to 0.2µm using Whatman membranes. Initial measurement of pH for each filter fraction was not routinely carried out in this work, but for the two samples which were analysed show only a marginal change in pH after filtration (unfiltered (7.82 and 7.91), 1.2µm (7.9 and 7.93), 0.1µm (8.07 and 8.08)). For this reason changes in pH are presented as a result of chemical or biological, rather than physical, change.

In this work significant changes in pH are only associated with samples stored at 11°C in light/ dark cycles. Larger changes in pH are recorded in the unfiltered samples than other fractions. Increases in pH of +0.57 to +3.81 pH units were observed in unfiltered fractions of all samples, of -0.26 to +2.56 pH units in 1.2µm filtered samples and -0.15 to +3.78 pH units in 0.1µm filtered. Changes in pH in pH units over 600 hours of sample storage at 11°C in light/ dark cycles are presented in Table 5.8.

Table 5.8: Change in pH (pH units) in unfiltered, 1.2µm and 0.1µm fractions of River Dove, Repton Brook, River Rea, River Tame and Bourne Brook samples between 0 and 600 hours after sample collection and storage at 11°C in light/ dark cycles.

	Unfiltered	**1.2µm**	**0.1µm**
Sample Name	+/-	+/-	+/-
River Dove	+2.55	+2.26	+2.07
Repton Brook	+0.57	-0.26	+0.32
River Rea	+0.48	+0.44	+0.31
River Tame	+3.81	+2.56	+3.78
Bourne Brook	+3.54	-0.38	-0.15

Potential changes in peaks A and C are modelled using the data of Patel-Sorrentino et al., (2002) in which four natural river waters were filtered to 1.2µm and 0.2µm and the pH of the filtered fractions artificially altered to a range of values from pH 2-12. Samples were fluoresced and changes in peak A and C fluorescence attributed to pH change.

Using data from Patel-Sorrentino et al., (2002) the fluorescence intensity of peaks A and C were plotted against pH for pH 6, 8 and 10 (which are relevant to the range of pH observed in this work) for each river and the mean gradients of the regression lines for peaks A and C were determined. For peak A the mean gradients of the regression lines for all river waters were 11.9 for samples filtered to 1.2µm and 7.6 for samples filtered to 0.2µm. For peak C the mean gradients of the regression lines for all waters were 1 for samples filtered to 1.2µm and 2.6 for samples filtered to 0.2µm. To study the potential influence of pH upon peak A and C fluorescence intensity a range of gradients around this value are used as correction factors (-3, 0, +3 peak C) (+5, +10, +15 peak A). The gradient of unfiltered samples is applied to

unfiltered and 1.2µm fractions and the gradient of samples filtered at 0.2µm is applied to 0.1µm fraction. Any additional change from the modelled value is proposed to be caused by microbial or photochemical change.

In line with the findings of Patel-Sorrentino et al., (2002) Peak A is more reactive to pH change than Peak C. The percentage of observed fluorescence change which may be a result of pH change for Peak C is shown in Table 5.9. The percentage of observed fluorescence change which may be a result of pH change for Peak A is shown in Table 5.10.

Table 5.9: The percentage of observed fluorescence intensity change which may be a result of pH change for Peak C, a result of modelling using the change in fluorescence intensity after manufactured pH change in the work of Patel-Sorrentino et al., (2002) as a baseline change.

	Unfiltered			**1.2µm fraction**			**0.1µm fraction**		
Peak	**C**			**C**			**C**		
mean gradient		**1**			**1**			**2.6**	
Correction factors	**-3**	**0**	**3**	**-3**	**0**	**3**	**0**	**2.5**	**5**
R. Dove	76	82	89	85	91	98	87	92	98
Repton Br.	84	86	88	92	91	90	77	78	79
R. Rea	83	84	86	90	92	93	95	96	97
R. Tame	115	130	145	99	108	117	123	135	147
Bourne Br.	96	102	109	106	105	104	105	105	105

Table 5.10: The percentage of observed fluorescence intensity change which may be a result of pH change for Peak A, a result of modelling using the change in fluorescence intensity after manufactured pH change in the work of Patel-Sorrentino et al., (2002) as a baseline change.

	Unfiltered			**1.2µm fraction**			**0.1µm fraction**		
	A			**A**			**A**		
mean gradient		**11.9**			**11.9**			**7.6**	
Correction factors	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**7.5**	**10**
R. Dove	76	81	85	77	81	85	81	83	85
Repton Br.	85	86	87	91	90	89	77	77	77
R. Rea	68	69	69	74	75	76	84	84	84
R. Tame	87	93	99	108	113	118	121	125	129
Bourne Br.	93	96	100	94	94	93	105	105	105

This model shows that a large percentage of the fluorescence intensity increase observed in peaks A and C in samples stored at 11°C in light/ dark cycles is due to pH change. In general, over 80% of peak C fluorescence increase is likely to be due to pH change while over 60% peak A fluorescence change is likely to be a result of pH change. However, allowing for inaccuracies in the model this does suggest some of the change in humic-like fluorescence in some samples is due to other factors. These values were calculated after application of the correction factors to all fluorescence measurements, even those for which no significant change in pH was observed.

It was not possible to develop a model to assess the degree of change in tryptophan-like fluorescence intensity which results from pH change as no directly comparable work exists. However Baker et al., (2007) found that T_2 fluorescence intensity decreased greatly in samples which were filtered and

had ambient pH manually adjusted by +/- 2pH units. In samples filtered to 1.2µm change in fluorescence intensity of <40% occurred when pH was altered by +/-2 units. In samples filtered to 0.2µm fluorescence changes of <30% occurred when pH changed by +/- 2 units. Reynolds, (2003) identified that in tryptophan standards of pH < 4.5 fluorescence decreased by up to 15%, pH 5-8 there was little impact, and at >pH 8 fluorescence was enhanced by up to 30%. Spencer et al., (2007) report that T_1 fluorescence intensity and peak position demonstrated no response to changing pH over the pH2-10 range.

In this work the response of T_1 and T_2 fluorescence intensity is far in excess of that reported in previous works. This may be a result of the range of pH being in excess of those reported by Baker et al., (2007) and the samples being natural waters with active biological systems rather than tryptophan standards as investigated by Reynolds, (2003). Percentage changes in the fluorescence intensity of peaks T_1 and T_2 over 600 hours are shown in Table 5.11, with changes in pH in pH units for each fraction of each sample stored at 11°C in light/ dark cycles.

Table 5.11: A summary of the change in mean tryptophan-like fluorescence intensity (as a percentage of original value) and pH change (pH units) between 0 and 600 hours for unfiltered, 1.2µm and 0.1µm filtered fractions of River Dove, Repton Brook, River Rea, River Tame and Bourne Brook samples.

Sample	Fraction	pH change (%)	T_1 intensity change (%)	T_2 intensity change (%)
River Dove	Unfiltered	+2.55	+122	+75
Repton Brook	Unfiltered	+0.57	+27	+1
River Rea	Unfiltered	+0.48	+42	+40
River Tame	Unfiltered	+3.81	-24	-22
Bourne Brook	Unfiltered	+3.54	+56	+30
River Dove	1.2µm	+2.26	+293	+237
Repton Brook	1.2µm	-0.26	-11	-32
River Rea	1.2µm	+0.44	+37	+53
River Tame	1.2µm	+2.56	+11	-4
Bourne Brook	1.2µm	-0.38	-17	-3
River Dove	0.1µm	+2.07	+346	+248
Repton Brook	0.1µm	+0.32	+138	+65
River Rea	0.1µm	+0.31	+47	+46
River Tame	0.1µm	+3.78	+17	-2
Bourne Brook	0.1µm	-0.15	-7	+7

There is no clear pattern of behaviour or magnitude of the fluorescence response to changing pH which appear to be specific to individual water samples. However it may be summarised that, in the majority of cases, when pH increases T_1 and T_2 fluorescence intensities also increase. Peak T_1 usually shows a greater increase in intensity than peak T_2. Increases in intensity in 0.1µm and 1.2µm fractions may be greater than the increases observed in the unfiltered fraction which may represent contamination of the sample with active biological material during the filtration process.

There is no consistent ratio of peak change between peaks T_1 and T_2 across this group of samples suggesting that, while they may be related, they act

independently and the changes that they undergo are specific to the chemistry of the sample. This may be a function of the position of the fluorophores on organic molecules and their degree of exposure to changes in the media.

5.3.5. The Effect of Light

Different patterns of behaviour are observed between samples stored under 11°C light/ dark cycles and 11°C dark conditions (which acts as a control for the samples stored in light/dark cycles). This does not take into account changes in these samples as a result of pH change (which it has been previously stated is difficult to quantify for tryptophan as no working models currently exist).

Direct comparison of fluorescence intensity values between samples stored at 11°C in light/ dark cycles and dark only conditions are summarised in Table 5.12. Unfiltered samples are identified as “Unf”. Data excludes samples of River Dove for which no dark control was kept. As CFU are lower in samples stored in light/ dark cycles than those stored at the same temperature in the dark it may be deduced that the increase in tryptophan-like intensity is due, not to biological activity but to pH effects and/ or photochemical transformation of the organic material present.

Table 5.12: Percentage change (mean of all samples - River Dove, Repton Brook, River Rea, River Tame and Bourne Brook) in fluorescence intensity for peaks T_1, T_2, C and A and water chemistry (Dissolved Oxygen (DO), pH, Conductivity (Cond), Total Organic Carbon (TOC), Total Carbon (TC) and Total Inorganic Carbon (TIC)) of unfiltered, 1.2µm and 0.1µm fractions between 0-600 hours for samples stored at 11°C in light / dark cycles and 11°C in the dark to determine the influence of light on sample stability during sample storage. Also presented are the total numbers of colony forming units (CFU) recorded after 600 hours.

		Fluorescence Intensity (AFU)				**Water Chemistry**						**CFU**	
	Dark or Light/ Dark	Peak T_1	Peak T_2	Peak C	Peak A	DO mg/L^{-1}	pH	Cond. µs/cm	TOC mg/L^{-1}	TC mg/L^{-1}	TIC mg/L^{-1}	YEA	R2A
		%	%	%	%	%	%	%	%	%	%	$CFUml^{-1}$	$CFUml^{-1}$
Unf	D	-2	-25	+3	+3	-19	-2	+5	-37	-10	-4	320000	163250
Unf	L/D	+25	+12	+2	+26	+43	+27	-13	+73	-57	-75	109000	48500
1.2µm	D	-10	-17	+3	+5	-17	-1	+4	-89	-2	-2	49250	108500
1.2µm	L/D	+5	+4	+2	+9	+36	+8	-3	-45	-22	-32	34750	6000
0.1µm	D	+4	-10	+4	+6	-13	+2	+5	-60	+1	-3	5250	10250
0.1µm	L/D	+49	+29	0	+9	+82	+14	-8	-14	-32	-5	6250	750

The results presented in Table 5.12 do not take into account changes in sample fluorescence as a result of pH change (discussed in section 5.3.4) or analytical uncertainty. Changes are presented as percentage change from the initial value recorded at hour 0. Between hours 0 and 600 samples stored at 11°C in the dark show a decrease in dissolved oxygen concentration, little change in pH, a slight increase in conductivity, a large decrease in TOC suggesting that dissolved organic carbon is being consumed. A decrease in TC and little change in TIC are also observed. Sample stored at 11°C in light / dark cycles increase in dissolved oxygen and pH but decrease in conductivity, TOC (except in the particulate fraction in which aggregation of dissolved and colloidal fractions to larger particulates in response to water chemistry change may be occurring), TC and TIC.

The CFU count in samples stored at 11°C in light/ dark cycles is often lower than in samples stored in 11°C dark conditions. The presence of a CFU count in the 0.1µm filtered fraction suggests that not all microbes were removed by filtration or that the filtration process (including filter unit cleaning prior to filtration) or plating technique were not sufficiently aseptic. Changes in fluorescence intensity (particularly peaks T_1 and T_2 which are not corrected for pH) do not relate well to CFU counts and therefore indicate that the bacterial community present in these samples is probably not the source of the fluorescence increases. It may also suggest that the growth of the bacterial community present in these river water samples is inhibited by light, and may only occur, or occur at a constant rate, during the dark cycles of the light/ dark storage cycle.

The effect of light upon excitation/ emission wavelength maxima is presented in Table 5.13 and Figure 5.2 a and b for samples stored at 11°C in dark only and light/ dark cycle conditions. Peak position change is low in most cases with notable exceptions being peak T_1 emission (red-shift) in unfiltered samples stored in the dark, T_1 and T_2 emission in 0.1µm fractions (blue-shift) and peak C excitation (red-shift) samples stored in dark and light/ dark cycles. The red-shift in peak C would support the hypothesis of an aggregation of dissolved and colloidal material to larger, less compact molecules in which lower excitation energy is required and greater internal reabsorption occurs.

Table 5.13: Change in excitation and emission wavelength (nm) in unfiltered, 1.2µm and 0.1µm filtered fractions (mean of the five surface water samples – River Dove, Repton Brook, River Rea, River Tame and Bourne Brook) observed in peaks T_1, T_2, C and A after 600 hours in samples stored at 11°C in light / dark cycles and 11°C in the dark.

		Excitation/ Emission Wavelength							
Fraction	Temp	Peak T_1		Peak T_2		Peak C		Peak A	
		ex	em	ex	em	ex	em	Ex	em
Unfiltered	11D	-2	+10	0	0	+7	0	-1	+2
1.2µm	11D	0	-2	+1	-6	+4	-2	+1	+3
0.1µm	11D	0	+2	-1	-3	+6	+1	-2	+2
Unfiltered	11L/D	-1	0	-6	-1	+10	-8	-11	-8
1.2µm	11L/D	+1	-3	-2	-5	+2	-5	-3	-5
0.1µm	11L/D	-3	-13	-2	-15	+9	+2	-4	+1

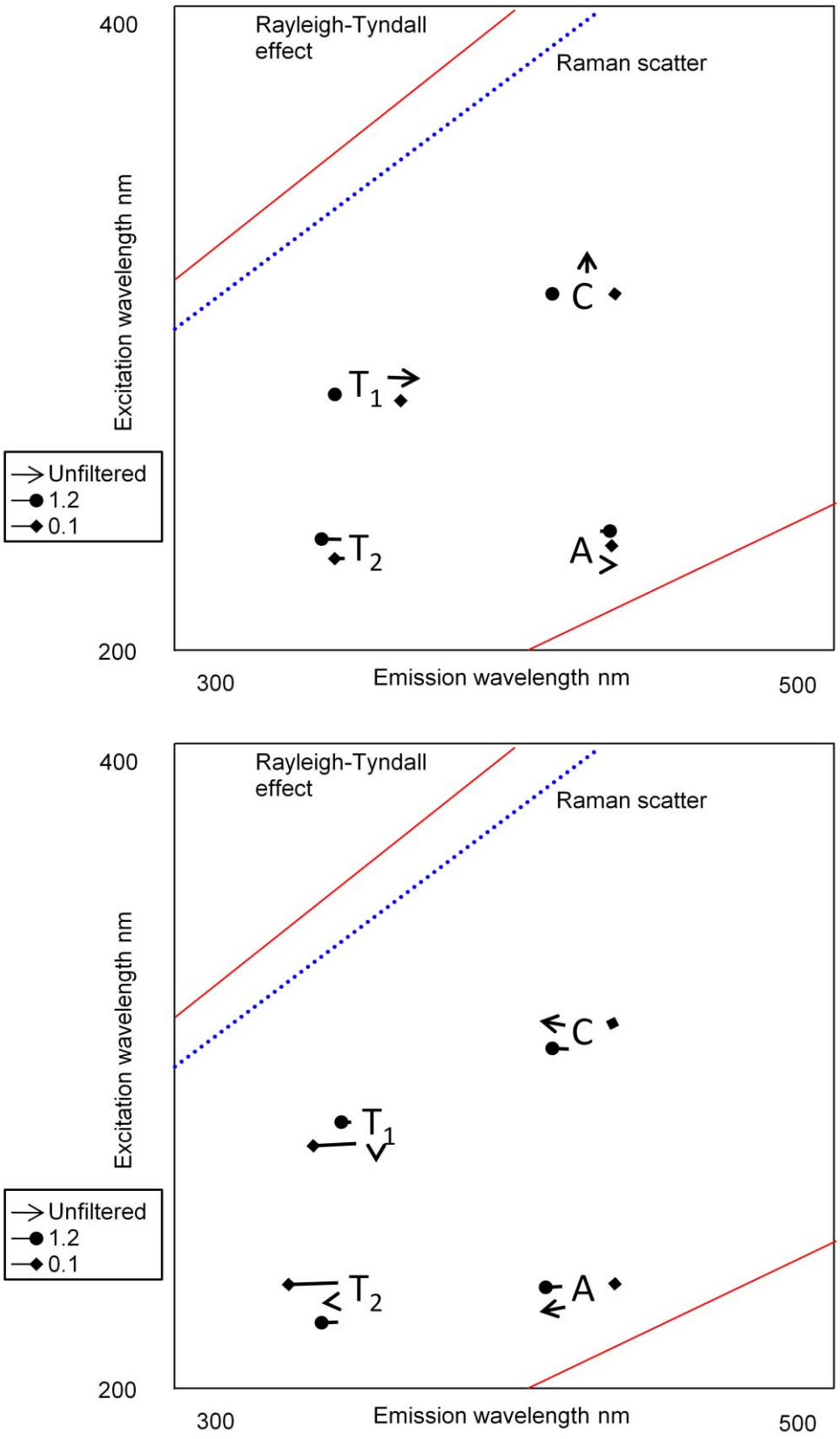


Figure 5.2a-b :The degree and direction of change for each fluorophore in samples stored at 11°C in the dark and 11°C in cycles light/ dark respectively

5.3.6. The Effect of Temperature

Fluorescence and water chemistry values are compared for samples stored under dark only conditions at 4°C, 11°C and 20°C as this excludes any changes caused by photodegradation of the sample. In addition any changes in fluorescence and water chemistry are not a result of pH change as there is no significant change in pH in these samples. Reported values are averages for all samples except River Dove (in the case of 11°C dark as no samples were kept under these conditions). Also reported in this section are counts of colony forming units to evaluate whether storage temperature plays a part in bacterial abundance and whether this may be a contributory factor to changes in fluorescence and water chemistry.

Table 5.14: Percentage change (mean of all samples - River Dove, Repton Brook, River Rea, River Tame and Bourne Brook) in fluorescence intensity for peaks T_1, T_2, C and A and water chemistry (Dissolved Oxygen (DO), pH, Conductivity (Cond), Total Organic Carbon (TOC), Total Carbon (TC) and Total Inorganic Carbon (TIC)) of unfiltered, 1.2µm and 0.1µm fractions between 0-600 hours for samples stored at 4°C in the dark, 11°C in the dark and 20°C in the dark to determine the influence of temperature on sample stability during sample storage. Also presented are the total numbers of colony forming units (CFU) recorded after 600 hours.

		Fluorescence Intensity (AFU)				**Water Chemistry**						**CFU**	
		Peak T_1	Peak T_2	Peak C	Peak A	DO mg/L^{-1}	pH	Cond. µs/cm	TOC mg/L^{-1}	TC mg/L^{-1}	TIC mg/L^{-1}	YEA	R2A
		%	%	%	%	%	%	%	%	%	%	$CFUml^{-1}$	$CFUml^{-1}$
Unf	4°C dark	+2	-19	+4	+5	-8	0	+3	+25	-5	-4	53400	74000
Unf.	11°C dark	-2	-25	+3	+3	-19	-2	+5	-37	-10	-4	320000	163250
Unf	20°C dark	+4	-19	+15	+19	-26	-6	+4	+10	-5	-2	390000	818000
1.2µm	4°C dark	-4	-12	+3	+5	-6	+1	+3	+5	0	-3	27000	27600
1.2µm	11°C dark	-10	-17	+3	+5	-17	-1	+4	nd	nd	nd	49250	108500
1.2µm	20°C dark	+1	-7	+11	+15	-25	-5	+4	-9	0	-2	236000	808000
0.1µm	4°C dark	+13	+2	+6	+8	-8	+3	+3	nd	nd	nd	2000	3600
0.1µm	11°C dark	+4	-10	+4	+6	-13	+5	+5	nd	nd	nd	5250	10250
0.1µm	20°C dark	+21	+5	+16	+22	-23	+4	+4	nd	nd	nd	41000	43600

As illustrated in Table 5.14 peak T_2 changes > +/- 10% between 0 and 600 hours under most conditions. Peaks C and A demonstrate the greater change in fluorescence intensity in samples stored at 20°C. Peak T_1, however, only demonstrates notable change in fluorescence intensity in filtered fractions, suggesting some degree of cell lysis. In general, dissolved oxygen concentrations decrease with time and to a greater extent in those samples stored at higher temperatures. pH remains consistent while conductivity increases to approximately the same extent regardless of storage temperature.

The CFU is greater in samples stored at higher temperatures with the highest CFU count always in samples stored at 20°C. Unfiltered fractions contain higher CFU counts than 1.2µm fractions, which contain more CFU than 0.1µm fractions. This supports the hypothesis that the common fluorophores, certainly T_1, C and A are related in some degree to the microbiological community with all of these peaks showing the greatest increase in intensity in samples stored at 20°C (the condition under which the microbial community is incubated during the BOD_5 test). In addition it suggests that a degree of fluorophore production is photo-independent.

Table 5.15: Change in excitation and emission wavelength (nm) in unfiltered, 1.2µm and 0.1µm filtered fractions (mean of the five surface water samples – River Dove, Repton Brook, River Rea, River Tame and Bourne Brook) observed in peaks T_1, T_2, C and A after 600 hours in samples stored at 4°C in the dark, 11°C in the dark and 20°C in the dark.

		Excitation/ Emission Wavelength							
Fraction		Peak T_1		Peak T_2		Peak C		Peak A	
		ex	em	ex	Em	ex	em	Ex	em
Unfiltered	4°C	-1	+11	0	+1	+6	+1	-1	+1
Unfiltered	11°C	-2	+10	0	0	+8	0	-1	+2
Unfiltered	20°C	-3	+10	+1	+5	+10	+2	-2	+5
1.2µm	4°C	0	-1	-1	-6	0	-4	0	+4
1.2µm	11°C	0	-2	+1	-6	+4	-2	+1	+3
1.2µm	20°C	0	+3	-1	-4	+7	0	-1	+4
0.1µm	4°C	0	+2	+3	-1	+6	+3	-1	0
0.1µm	11°C	0	+2	-1	-3	+6	+2	-2	+2
0.1µm	20°C	1	0	-1	-6	+12	+9	-2	+6

Excitation/ emission maxima changes are presented in Table 5.15 for samples stored in the dark in a range of temperatures. In this instance changes in excitation wavelength are generally very low except peak C under all conditions which red-shifts. Degree of red-shift is usually higher in samples stored at higher temperatures. Emission wavelength generally changes to a greater extent with the predominant change being a red-shift.

5.3.7. Onset of Algal Growth

Algal growth was observed on the walls of the storage bottles in unfiltered samples stored under 11°C light/ dark conditions. The species of algae was not determined and the appearance of the algae was either green tendrils, or a brown dusty wall covering. The algal growth was observed 72 hours after

sample collection in the River Rea and River Tame samples and after 264 hours in River Dove, Repton Brook and Bourne Brook samples.

5.4. Discussion

When the impact of light exposure and temperature upon fluorescence intensity and water chemistry are assessed using ANCOVA (ANalysis of COVAriance) models it is evident that (in these samples) peaks T_1 and T_2 and all water chemistry parameters are subject to change when samples are exposed to light during storage. Peaks C and A, however are more significantly affected by storage at different temperatures. In samples exposed to light filtration has significant effect upon samples during storage while those samples exposed to different storage temperatures are generally more stable regardless of filtered fraction although pH, conductivity and TOC demonstrate significant change between filtered fractions. Within these broad summaries significant differences between means have been found for almost all parameters between the unfiltered and 1.2µm fractions and the 1.2µm and 0.1µm fractions under all conditions of light and temperature. Furthermore there are significant differences between mean tryptophan-like fluorophores in samples stored in the dark at 11°C and 20°C in which mean fluorescence intensities in samples stored at 20°C are significantly lower. Humic-like peaks C and A, conversely, demonstrate significant differences between samples stored in the dark at 4°C and 11°C in which mean fluorescence intensities in samples from 11°C are significantly higher.

Thus it may be surmised that storage temperature has less impact upon sample stability than exposure to light. Filtration has a significant effect upon the response of samples to different storage conditions and within the general results other trends in fluorescence and water chemistry change by storage condition may be observed.

On a river by river basis peaks A and C tend to increase in fluorescence intensity over time with peak A usually demonstrating a greater degree of change than peak C. Peak T_1 remains steady in most fractions and samples, except 0.1µm fractions in which an increase is commonly observed which may be a result of cell damage during vacuum filtration, a microbial product or material which can pass through a 0.1µm filter. Peak T_2 intensity decreases with time under most storage conditions, demonstrating that the two tryptophan-like peaks do not operate in tandem; rather they are independent entities which may be a result of different sources or positions within molecular structures. However, at 11°C in light/ dark cycles it is most common to see a dramatic increase in peak T_1 and T_2 fluorescence intensities.

Peaks T_1, T_2 and A all demonstrate shifts to shorter excitation and emission wavelengths (blue-shift) in samples stored at 11°C light/ dark suggesting that humics may be becoming less aromatic, all fluorescent compounds are becoming smaller or that concentration of fluorophores may be decreasing. Peaks C and A in samples stored at 20°C dark tend to move to longer emission wavelengths (red-shift) which may indicate an increase in

hydrophobicity, molecular size or a concentration of fluorescent compounds increasing inner filtering.

At higher temperatures in the dark it is likely that the labile (T_1 and T_2) portion of TOC is being oxidised by the microbial community, producing degraded humic like material (peaks C and A). However, no new organic material is formed in most cases (with the exception of River Dove and Repton Brook). In samples stored at 11°C in light / dark cycles the changes in water chemistry indicate active photosynthesis by algae, with dissolved oxygen increasing as a by-product of CO_2 use during respiration. As CO_2 and HCO_3 are taken in and processed the alkalinity of the water increases and TOC, TC and TIC concentrations decrease. TOC increases in the unfiltered fraction possibly indicate that some dissolved and colloidal organic carbon is aggregating to larger particulates as a result of water chemistry change, supported by a red-shift in Peak C excitation (strongly correlated with organic carbon concentration). However, it is also possible that the changes in fluorescence intensity observed may be a result of excretion of microbial products including labile T_1 and T_2 fluorescent material (probably proteins and lipids) and humic-like material (peaks A and C), both of which may include dead cell material.

All of the changes described above should be treated with an element of caution as some of the fluorescence intensity changes observed in these stored samples could be explained by analytical uncertainty inherent in the fluorescence method. All fluorescence data for samples stored at 4°C dark and 11°C dark may be ascribed to analytical uncertainty. In addition changes

in peaks T_1 and T_2 which occur in samples stored at 20°C dark may be ascribed in the same way. The remaining parameters (changes in peaks T_1, T_2, C and A in samples stored at 11°C light/ dark and peaks A and C in samples stored at 20°C dark) may be classed as actual observed fluorescence change.

However, of this observed change up to 80% fluorescence intensity change in peak C and 60% of peak A observed in samples stored at 11°C light/ dark may be attributed to molecular conformation change as a result of changing pH. When this is taken into account alongside the issue of analytical uncertainty all change in peak C intensity in 11°C light/ dark samples becomes a matter of analytical uncertainty, while peak A retains its status of observed actual change. All changes in peaks C and A that are observed in samples from 20°C dark conditions remain actual observed changes as there is no consistent pH change against which to model pH influence at this temperature.

5.5. Summary

1. Light exposure has a significant effect upon sample stability in this work but storage temperature is less influential in sample change. It is likely that changes in fluorescence intensity and water chemistry in samples exposed to light are probably also influenced by other factors such as algal growth (which may be influenced by light and/ or temperature) which has not been analysed in detail in this work.

2. When possible analytical uncertainty as derived in this chapter is considered the only changes in fluorescence which may be considered to be “actual” change are those in peaks T_1, T_2, C and A in samples stored at 11°C light/ dark and peaks C and A stored at 20°C dark. When the influence of changing pH upon fluorescence intensity, as modelled in this chapter, is considered the vast majority of peak C change in samples stored at 11°C light/ dark becomes attributable to analytical uncertainty.

3. As there not notably higher bacterial growth in samples stored at 11°C light/ dark compared with those stored at 11°C dark it is considered that the origin of the fluorescence intensity increase is not bacterial activity. Rather it is attributed to photosynthetic activity by algae in samples stored at 11°C light/ dark.

4. Water chemistry changes support the theory that increases in fluorescence intensity in samples stored at 11°C light/ dark are a result of photosynthetic activity even in 0.1µm filtered fractions This suggests that filtration to 0.1µm does not remove all photosynthetic microorganisms from the sample, so should not be considered to be a method of sample sterilization. Dissolved oxygen and organic carbon concentrations are seen to increase as a product of the respiration of inorganic carbon, the removal from solution of which decreases solution pH to strongly alkaline levels.

5. Increases in peak C and A fluorescence in samples stored at 11°C and 20°C in the dark are likely to be a result of microbial processing of labile organic carbon and dead cell material from the bacterial/ algal cells present.

6. Peaks C and A often behave in similar manners demonstrating similar directions and magnitudes of change, although the change in excitation/ emission wavelength maxima is not always consistent. However, peaks T_1 and T_2 often display different directions and magnitudes of change in both intensity and excitation and emission wavelength. In line with the findings of other chapters this suggests that peaks T_1 and T_2 are subject to different mechanisms of change related to their source, character or molecular position.

7. Recommendations for sample storage protocols are that samples should be kept in the dark under cool conditions. As algal cells vary in shape and dimension in all directions even samples filtered to 0.1µm may not be totally sterile so even under these rigorous conditions some mineralisation of organic carbon should be expected. This varies on a river by river basis and thus it is not possible to present a guide to expected degree of sample change.

6. CHANGES IN FLUORESCENCE WITH FREEZING/ THAWING AND DEHYDRATION/ REHYDRATION

Rationale

How the fluorescence intensity of fresh water organic matter changes with cycles of dehydration/ rehydration and freezing/ thawing as a laboratory-scale proxy for these events in the environment and to inform the debate on sample storage and stability.

6.1. Introduction

As described in Chapter 2 the basic fluorescence methods undertaken in this chapter are the same as in previous chapters. In addition samples were analysed for a suite of common freshwater parameters by the Environment Agency to allow an assessment of the influence of initial sample character upon the type and degree of change observed.

In this chapter fluorescence excitation emission matrices (EEMs) and water chemistry parameters are interrogated to determine the stability of river water and lake water samples under conditions which may be experienced in the environment and which are, or could be, used for sample preservation after collection. All fluorescent peaks are of interest as their behaviour under extremes of temperature and desiccation should tell us something about the behaviour of the molecules under those conditions.

Freezing is a common method of sample storage, particularly when long field trials are undertaken and analysis cannot be completed for some time. This chapter aims to investigate the stability of sample fluorescence under frozen and dehydrated conditions to consider whether these are suitable methods for sample preservation. In addition, periods of freezing and desiccation may occur seasonally in the natural environment. In some way this chapter aims to address the issue of what happens to the fluorescent organic carbon during these processes, in an attempt to contribute to the issues of sample stability and carbon budgets within the aquatic environment.

The conditions under which samples were frozen, dried and thawed, rehydrated were chosen to be sustainable within the laboratory for prolonged periods. They were also such that a full cycle of freezing/ thawing and dehydration/ rehydration would not take longer than a few days to allow multiple cycles of analysis to be undertaken in the time available.

6.2. Literature Review

Differing results have been obtained regarding the effect of freezing and thawing upon samples stored for fluorescence analysis. Many works have used freezing as a method of sample preservation and it is commonly used in studies in which sampling is carried out over a prolonged period of time, or at a distance from the analysis site, and is particularly common in studies of marine waters (Coble, 1996; Del Castillo et al.,1999). Frozen samples are occasionally used as reference samples for analysis of changes in non frozen

samples under different conditions of mixing, time, photo degradation for example in work on marine waters by Coble, (1996), and surface waters Wiegner and Seitzinger, (2001) but commonly with no clear assessment of the potential impact of the freezing process upon the frozen sample character e.g. organic matter and nutrient concentrations, fluorescence properties.

However, a number of works have investigated the stability of nutrients in frozen samples. In storage stability trials using freezing Avanzino and Kennedy (1993) identified that in stream waters orthophosphate and ammonia levels declined while nitrate/ nitrite levels increased but the scale of change was no more than 2 standard deviations of the method error and thus the nutrients were essentially unaffected. Dore et al., (1996) determined by comparison of nitrate/ nitrite and silicate that the relationship between fresh and frozen samples in marine waters is a perfect r=1, suggesting no impact on nutrient levels through the freezing process.

More recently Fellman et al., (2008) concluded that freezing of surface waters leads to a decrease in dissolved organic nitrogen (DON), dissolved organic carbon (DOC) and dissolved organic phosphate (DOP). In addition to this they summarised that as the specific UV absorbance (SUVA) of the samples decrease after freezing thus aromatic dissolved organic carbon was being preferentially removed by the process. While the change in DON and DOP would not be reflected by fluorescence spectroscopy, the decrease in aromatic DOC should, by a change in peak position and or a decline in fluorescence intensity. Reemtsma and Jekel (1997) and Fellman et al., (2008) identified that precipitates often form during the freezing process in tannery

wastewaters and surface waters respectively. The settlement of these particles during the DOC analysis process could be the reason for DOC loss after freezing if freezing causes aggregation of organic carbon particles.

Komada et al., (2002) and Mayer et al., (2006) find that similar patterns in fluorescence or DOC change are observed in river and coastal waters regardless of the way in which samples are handled during preservation and preparation. Contrary to these findings, and in a direct assessment of the effect of sample freezing upon fluorescence, Spencer et al., (2007) propose that surface water fluorescence peak positions and intensities are affected by the freezing process and that the random nature of the changes makes freezing an unsuitable method of sample preservation for fluorescence analysis.

Spencer et al., (2007) found that the excitation and emission wavelengths of specific peaks changed after freezing and thawing, but that the degree of change was within the bounds of method error. However, fluorescence intensity changed dramatically but inconsistently across the common peaks, with 80% samples changing to a greater extent than the error of method reproducibility. Changes included both increases and decreases and had no clear association to original sample chemistry or character. This suggests that for the upland waters analysed in that paper freezing is an unsuitable method of sample preservation.

No works have been found to date that assess the impact of dehydration and rehydration of liquid samples upon their intrinsic fluorescence. Many soil science papers investigate the changes in organic matter extracted from soils after the drying process as land desiccation is a common problem in many parts of the world, and the drying of soils affects the rate and type of organic matter released upon rehydration. Thus this work has an impact upon hydrological fluorescence, but the results of these studies are not directly relevant to this work.

It is proposed that the age and degree of degradation of aquatic organic matter by photo- or biological processes are likely to affect the degree of change in organic matter fluorescence as a result of freezing and thawing or dehydration and rehydration. The literature cited above indicates that marine organic matter, much of which is old, highly degraded terrestrial material, is relatively stable upon freezing and thawing. It may be hypothesised that "younger" organic matter e.g. microbial products, humic material from peatlands and other terrestrial humics in river waters are more likely to demonstrate changes in fluorescence intensity as a result of changes in molecular confirmation through the freeze/ thaw and dehydration/ rehydration processes. It is possible that the extent of change may be greater for those peaks which are considered to be representative of more labile fractions of aquatic organic matter i.e. tryptophan-like fluorescence centres. While those peaks which represent more stable, degraded materials i.e. humic-like peaks A and C may show a smaller degree of fluorescence intensity variability. However, freezing of organic matter has been shown to cause aggregation

(Fellman et al., 2008). Settlement of aggregated particles may have a significant effect in reducing fluorescence intensity.

This chapter assesses the impact upon river water fluorescence of the freezing/ thawing and dehydration/ rehydration processes with a view to determining whether freezing is a suitable method of sample preservation for such samples. It also aims to indicate changes that may be expected in fluorescent organic matter in the environment in freeze/ thaw and dehydration/ rehydration events. The findings of this chapter may also be found in Hudson et al., (2009).

6.3. Description of Samples and Sample Preparation

6.3.1. Description of Samples

Samples were collected from 13 surface water sites in two batches during November 2006 and January 2007. The waters collected are a combination of rural, sub-urban and urban surface waters with varying degrees of channel modification. During sample collection periods flow rates were high with some watercourses being in flood. Samples are ordered from most urban to most rural character. The water chemistry values recorded by the Environment Agency and TOC and initial fluorescence intensity values (in AFU) measured at the University of Birmingham are presented in Tables 6.1and 6.2.

Table 6.1: Water chemistry values (Biochemical Oxygen Demand (BOD), Alkalinity (Alk), Ammonia (AmN), Chloride (Chl), Total Oxidised Nitrogen (TON), Nitrite (NO_2^-), Orthophosphate (Orthop), Silicate (SiO_2), Phosphate (Phosp), Conductivity (Cond), pH, Turbidity (Turb) and Nitrate (NO_3^-)) recorded after receipt of the samples at the laboratory by the Environment Agency for all samples to be analysed for fluorescence change after freezing/ thawing and dehydration/ rehydration.

Sample Name	I.D	BOD	Alk	AmN	Chl	TON	NO_2^-	Orthop	SiO_2	Phosp	Cond	pH	Turb	NO_3^-
		mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	µS/cm	units	FTU	mg/l
River Tame	1	<3.0	177	0.21	135	7.5	0.145	1.88	11.2	2.29	929	7.70	9.3	7.3
Wood Brook	2	25.9	160	7.47	35	3.0	0.759	0.90	10.6	1.20	479	7.61	14.5	2.2
River Rea	3	<3.0	158	0.06	80	2.8	0.055	0.13	11.6	0.13	682	7.95	1.4	2.7
Harborne Brook North	4	5.6	75	0.53	796	1.8	0.139	0.15	6.8	0.16	2490	7.05	10.5	1.7
Harborne Brook South	5	<3.0	87	0.16	627	1.9	0.068	0.16	7.1	0.16	2000	7.30	6.6	1.9
Vale Lake	6	<3.0	85	0.97	23	0.3	0.024	0.12	7.8	0.12	282	7.35	5.2	0.3
Bartley Brook	7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Merritt's Brook	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
River Trent	9	<2.9	111	0.28	41	7.1	0.093	0.54	9.8	0.66	512	7.68	54.3	7.0
Repton Brook	10	<3.0	225	<0.03	45	6.6	0.019	0.04	9.6	0.04	659	7.95	7.2	6.6
Hilton Brook	11	<2.9	135	0.13	22	6.9	0.044	0.28	9.2	0.34	421	7.82	62.5	6.8
River Dove	12	<3.0	160	<0.03	31	4.1	0.019	0.17	7.5	0.16	520	8.05	1.7	4.1
Alder Brook	13	<2.9	192	0.14	29	8.1	0.043	0.26	14.7	0.29	734	8.04	49.3	8.0
18MΩ Control		<3.0	<15	<0.03	<1	<0.2	<0.004	<0.02	<0.2	<0.02	<10	6.95	<1.0	<0.2

Table 6.2: Total Organic Carbon (TOC), Total Carbon (TC), Total Inorganic Carbon (TIC) and Peak T_1, T_2, C and A fluorescence intensity results for all samples measured on the day of collection prior to freezing or dehydration.

Sample Name	**I.D**	**TOC mg/l**	**TC mg/l**	**TIC mg/l**	**Fluorescence character recorded on Day 1**											
					Peak T_1			Peak T_2			Peak C			Peak A		
					Ex.	Em.	Int.	Ex.	Em.	Int.	Ex.	Em.	Int.	Ex.	Em.	Int.
River Tame	1	6.49	49.98	43.49	283	362	147	232	351	355	331	414	191	239	420	370
Wood Brook	2	ND	ND	ND	280	371	396	228	362	689	325	419	291	222	413	857
River Rea	3	3.64	43.68	40.04	285	350	69	232	349	224	330	420	146	237	407	319
Harborne Brook North	4	12.46	30.54	18.08	282	344	210	233	360	754	316	420	280	237	414	756
Harborne Brook South	5	9.71	31.19	21.48	281	342	134	231	345	534	300	416	220	231	407	554
Vale Lake	6	6.51	28.02	21.51	285	348	135	231	369	505	316	424	168	237	411	470
Bartley Brook	7	ND	ND	ND	282	344	64	230	342	149	335	421	197	231	425	417
Merritt's Brook	8	ND	ND	ND	282	344	83	233	350	197	341	419	305	236	430	493
River Trent	9	ND	ND	ND	280	344	99	233	356	252	332	422	324	238	415	560
Repton Brook	10	2.03	58.67	56.64	280	359	49	233	360	96	321	429	107	238	417	235
Hilton Brook	11	ND	ND	ND	285	355	136	227	348	340	331	421	488	236	429	868
River Dove	12	3.64	44.90	41.26	288	344	38	232	368	143	341	429	165	237	420	317
Alder Brook	13	ND	ND	ND	278	361	139	228	354	235	340	418	469	237	439	868
18MΩ Control		0.20	0.40	0.20	280	354	2	231	352	13	336	417	2	227	398	6

6.3.2. Collection and Preparation of Samples

Samples were collected and prepared for analysis as described in 2.4.4.2; 2.4.4.3 and 2.4.4.4.

6.4. Results

Further to the results within this chapter the analysis of data could be taken further by ANOVA, similar to the models presented in Chapter 5, treating the data as two separate experiments a) the effect of freezing and thawing on organic matter fluorescence and b) the effect of dehydration/ rehydration on organic matter fluorescence.

6.4.1. Data Correction and Analytical Uncertainty

Ten samples were analysed in triplicate in this section which is insufficient to enable a real assessment of deviation from mean data which could be considered to indicate analytical uncertainty. For this reason the value of one standard deviation from the mean of triplicate samples from Chapter 5 (424 samples) will be used to assess whether the change in fluorescence intensity observed may be reportable change in intensity or simply attributable to analytical uncertainty.

The maximum standard deviation value from all 424 samples is considered to represent the maximum variability across triplicate analysis thus changes in fluorescence intensity which are less than the maximum standard deviation value for the peak are considered to fall within the bounds of analytical

uncertainty. Changes in fluorescence intensity which exceed the maximum standard deviation value from triplicate analysis are considered to be actual changes in fluorescence character. A summary of the standard deviation around the mean as a value and as a percentage of the mean intensity for samples subjected to different storage conditions is shown in Table 6.3. Highlighted values are maximum standard deviation as a percentage of mean intensity.

Table 6.3: Summary of standard deviations for each fluorescence peak T_1, T_2, C and A (as actual values and percentage mean intensity) from the mean for 424 triplicate samples recorded in Chapter 5. To be used in the assessment of whether observed change in fluorescence intensity is an actual change or an artefact of analytical uncertainty.

Standard deviation	T_1		T_2		C		A	
	Actual	**%**	**Actual**	**%**	**Actual**	**%**	**Actual**	**%**
Minimum	0	0	0	0	0	0	1	0
Maximum	23	27	40	18	12	8	95	17
Mean	4	6	9	5	3	3	10	3

Change in fluorescence intensity is presented as a recorded value and as percentage change from the initial recorded value. Fluorescence intensity has been normalised to a constant Raman intensity and also corrected for changes in intensity in an18MΏ deionised water sample which was stored under the same conditions as the collected samples. The fluorescence intensity in AFU recorded for each peak in the 18MΏ deionised water sample was subtracted from the fluorescence intensity recorded for each peak in the frozen/ thawed and dehydrated/ rehydrated surface water samples. Changes in fluorescence intensity from initial values were calculated from the corrected

values. The purpose of this correction was to account for contaminants which could potentially enter the sample from the storage containers or from the air (during drying). On average peak T_1 was corrected by -4 AFU, T_2 by -43 AFU, C by -3 AFU and A by -21 AFU, minimal values (within instrument variability) except peak T2. Where no corresponding18MΏ deionised water sample was stored an average correction factor calculated from available 18MΏ deionised water sample data for each peak was applied (frozen samples of Alder Brook, Bartley Brook, Hilton Brook, Merritt's Brook, River Trent and Wood Brook).

6.4.2. Stability of Refrigerated Control Samples

A subsample of each surface water sample was stored under refrigerated conditions (4°C in the dark) for the duration of the freeze/ thaw and dehydration/ rehydration cycles. In Chapter 5 it was shown that although samples stored under refrigerated conditions are not totally stable and some change in fluorescence intensity is observed the vast majority of fluorescence change could feasibly be attributable to analytical uncertainty for all peaks.

In these surface waters the refrigerated control samples almost all changed to a degree which could not be attributed to analytical uncertainty, within the bounds of maximum standard deviation. This is illustrated in Table 6.4. Values highlighted indicate those outside the maximum range of analytical uncertainty.

Table 6.4: The change in fluorescence intensity observed for each fluorescence peak (T_1, T_2, C and A) for each of the samples as a percentage change (%) from the value recorded on Day 1 in refrigerated control samples.

Sample name	**I.D**	**Percent change fluorescence intensity**			
		T_1	T_2	C	A
River Tame	1	-19	11	15	28
Wood's Brook	2	-81	-64	-18	-52
River Rea	3	-36	-28	0	6
Harborne Brook North	4	-41	-42	-15	-16
Harborne Brook South	5	-20	-7	4	14
Vale Lake	6	-14	-28	12	9
Bartley Brook	7	12	-29	2	8
Merritt's Brook	8	-6	-44	-18	-2
River Trent	9	-34	-42	-14	-4
Repton Brook	10	-32	-62	7	-3
Hilton Brook	11	-59	-69	-22	-25
River Dove	12	-5	-49	-2	5
Alder Brook	13	-62	-61	-20	-16
Mean fluorescence change %		-31	-40	-5	0
Standard deviation		26	23	13	15

Peaks T_2 and C generally decrease by values which exceed analytical uncertainty shown in Table 6.3 indicating that under refrigerated conditions the fluorescent organic matter comprising these peaks is subject to oxidation or microbial degradation. Peak T_1 is less affected and one may surmise that peak A is quite stable under refrigerated conditions. If the results of freezing/ thawing and dehydration/ rehydration analysis demonstrate greater changes in fluorescence intensity than those observed in refrigerated controls it may be concluded that the processes of freezing/ thawing and dehydration/ rehydration are more influential as mechanisms of change in organic matter fluorescence than oxidation or microbial degradation.

6.4.3. Changes in Fluorescence Intensity after One Cycle of Freezing/ Thawing and One Cycle of Dehydration/ Rehydration

Samples were frozen and thawed five times to investigate the impact of the process upon fluorescence in the environment, in which multiple cycles of freezing and thawing may occur over a season. The results of this are discussed later in the chapter. Analysis of one freeze/ thaw event is pertinent to the question of sample storage as a number of studies use sample freezing as a method of preservation, particularly when long field seasons are undertaken. Results of one cycle of freezing and thawing are presented in Table 6.5. Highlighted values indicate those which fall outside the bounds of analytical uncertainty. It was not possible to undertake prolonged periods of frozen storage due to time constraints.

Table 6.5: Change in fluorescence intensity as a percentage of the initial value recorded on Day 1 (corrected for change in fluorescence intensity In an 18MΩ deionised water control sample) for peaks T_1, T_2, C and A after one freeze/ thaw cycle

Sample Name	**I.D**	**Percent change fluorescence intensity**			
		T_1	T_2	C	A
River Tame	1	-12	-26	-8	-6
Wood Brook	2	-12	-12	-10	-7
River Rea	3	19	-28	-11	-6
Harborne Brook North	4	11	-11	1	-2
Harborne Brook South	5	-5	-17	-7	-7
Vale Lake	6	-23	-35	-20	-30
Bartley Brook	7	19	-17	10	9
Merritt's Brook	8	-11	-14	-8	8
River Trent	9	-14	-29	-2	13
Repton Brook	10	2	-86	-4	-11
Hilton Brook	11	22	-51	-13	0
River Dove	12	2	-78	-13	-7
Alder Brook	13	-37	-37	-7	12
Mean fluorescence change %		-3	-34	-7	-3
Standard deviation		18	24	7	12

When maximum standard deviation is used as an identifier of analytical uncertainty almost all changes in peaks T_1 (-3 +/- 18%) and A (-3 +/- 12%) may be attributed to analytical uncertainty. This may be due to the difficulties sometimes associated with identifying the position of these peaks, when interference from Raman and Rayleigh-Tyndall scatter lines must be taken into consideration. Peaks T_2 and C are often more clearly observed, although peak T_2 may be obscured (as is the case in very clean samples such as the18MΩ deionised water) by interferences related to being excited at wavelengths close to the extremes of lamp output capacity in the instrument used. Changes in peak T_2 (-34 +/- 24%) and C (-7 +/- 7%) fluorescence more often fall outside the bounds of analytical uncertainty.

This suggests that while freezing may be a convenient method of fresh water sample storage it is not without detrimental effect on the fluorophores present. Substantial decreases in peak T_2 and C intensities, not purely attributable to analytical uncertainty, may be observed over a single episode of freezing and thawing even when frozen for a relatively short space of time.

Analysis of a single dehydration/ rehydration event may be pertinent to the question of sample storage as, although it is not currently used as a method of sample preservation, if good sample stability was shown dehydration would have the potential to be a useful storage technique for small volumes of sample. Freeze drying is currently used as a method of sample preservation, particularly in soil samples. However, this is a separate process and is likely to have different effects upon fluorescent organic matter to freezing, or drying.

Change in fluorescence intensity as a percentage of the intensity recorded on day 1 for each peak after one cycle of dehydration/ rehydration is presented in table 6.6. All presented data has been normalised against the water Raman and corrected as described in section 6.4.1. Highlighted cells indicate those in which values exceed analytical uncertainty as determined in section 6.4.1.

Table 6.6: Change in fluorescence intensity as a percentage of the initial value recorded on Day 1 (corrected for change in fluorescence intensity In an 18MΩ deionised water control sample) for peaks T_1, T_2, C and A after one dehydration/ rehydration cycle.

Sample Name	**I.D**	**Percent change fluorescence intensity**			
		T_1	T_2	C	A
River Tame	1	-30	-17	-15	-12
Wood Brook	2	-57	-55	-28	-33
River Rea	3	-23	-19	-19	-20
Harborne Brook North	4	-19	-29	-29	-30
Harborne Brook South	5	12	-12	-17	-14
Vale Lake	6		-22	-14	-21
Bartley Brook	7	-27	-55	2	1
Merritt's Brook	8	-42	-44	-29	-14
River Trent	9	-42	-53	-21	-8
Repton Brook	10	29	-52	-16	-26
Hilton Brook	11	-60	-59	-17	6
River Dove	12	-8	-67	-22	-21
Alder Brook	13	-40	-30	-15	-8
Mean fluorescence change %		-26	-40	-18	-15
Standard deviation		26	19	8	11

Like samples subjected to freezing and thawing, but to a greater extent, little fluorescence change in peaks T_2 and C (-40 +/- 19%) and (-18 +/- 8%) respectively. However, in samples subjected to dehydration and rehydration peaks T_1 and A also demonstrate changes which fall outside the range

specified for analytical uncertainty (-26 +/- 26%) and (-15 +/- 11%) respectively.

Thus, dehydration is not a suitable method of sample storage it has detrimental effect on the fluorescent organic matter present. Dehydration and rehydration is even more disruptive to sample fluorescence than freezing and thawing, indicated by more fluorophores in more samples falling outside the bounds of analytical uncertainty. This is relevant to the issue of sample stability and storage as it suggests that the structure of fluorescent organic matter in surface waters is effectively disrupted by dehydration.

6.4.4. Changes in Fluorescence Intensity after Five Cycles of Freezing/ Thawing and Five Cycles of Dehydration/ Rehydration

The results of fluorescence analysis on water subjected to five cycles of freezing/ thawing and dehydration/ rehydration are presented in Tables 6.7 and 6.8. All presented data have been normalised against the water Raman and corrected as described in 6.4.1. Highlighted cells indicate values that exceed analytical uncertainty as determined in 6.4.1. Full fluorescence excitation and emission wavelength and fluorescence intensity data by cycle of freezing and thawing and dehydration/ rehydration for each sample are presented in Appendices 5 and 6 respectively.

Table 6.7: Change in fluorescence intensity as a percentage of the initial value recorded on Day 1 (corrected for change in fluorescence intensity In an 18MΏ deionised water control sample) for peaks T_1, T_2, C and A after five freeze/ thaw cycles.

Sample name	**I.D**	**Percent change fluorescence intensity**			
		T_1	T_2	C	A
River Tame	1	-52	-28	-25	-28
Wood's Brook	2	-39	-33	-17	-33
River Rea	3	-46	-28	-31	-23
Harborne Brook North	4	-25	-11	-4	-14
Harborne Brook South	5	-33	-34	-36	-24
Vale Lake	6	-63	-53	-52	-44
Bartley Brook	7	-35	18	0	7
Merritt's Brook	8	-34	-22	-20	9
River Trent	9	-39	-8	-15	0
Repton Brook	10	-52	-78	-10	-17
Hilton Brook	11	-59	-57	-9	15
River Dove	12	-78	-90	-69	-65
Alder Brook	13	-43	-30	-13	11
Mean fluorescence change %		-46	-35	-23	-16
Standard deviation		15	29	20	24

Table 6.8: Change in fluorescence intensity as a percentage of the initial value recorded on Day 1 (corrected for change in fluorescence intensity In an 18MΏ deionised water control sample) for peaks T_1, T_2, C and A after five dehydration/ rehydration cycles.

Sample name	**I.D**	**Percent change fluorescence intensity**			
		T_1	T_2	C	A
River Tame	1	-44	-49	-32	-35
Wood's Brook	2	-77	-65	-29	-49
River Rea	3	-34	-63	-40	-37
Harborne Brook North	4	-3	-27	0	-7
Harborne Brook South	5	-67	-75	-67	-67
Vale Lake	6	-50	-52	-38	-39
Bartley Brook	7	-49	-60	-29	-25
Merritt's Brook	8	-51	-74	-32	-22
River Trent	9	-53	-63	-22	-16
Repton Brook	10	-32	-62	-30	-45
Hilton Brook	11	-67	-58	-33	-23
River Dove	12	-1	-78	-41	-36
Alder Brook	13	-70	-47	-30	-19
Mean fluorescence change %		-46	-59	-33	-32
Standard deviation		23	13	14	15

After five cycles of freezing / thawing and dehydration/ rehydration fluorescence intensity change becomes more consistent, with all peaks predominantly demonstrating a decrease in intensity. This trend is illustrated in Figures 6.1 and 6.2 in which the fluorescence intensity change for all peaks in all samples subjected to freezing/ thawing and dehydration/ rehydration respectively after one and five cycles are presented. Figures 6.1 and 6.2 also show that after five cycles of freezing/ thawing and dehydration/ rehydration fewer changes are attributable to analytical uncertainty than after one cycle of each process. Furthermore, the patterns observed are less clearly peak specific. Harborne Brook North (sample I.D 4) demonstrates contrary behaviour however Figure 6.3 shows that this sample also undergoes an initial decline in fluorescence intensity followed by an increase in intensity after cycle 3 in frozen samples and cycle 4 in dehydrated samples.

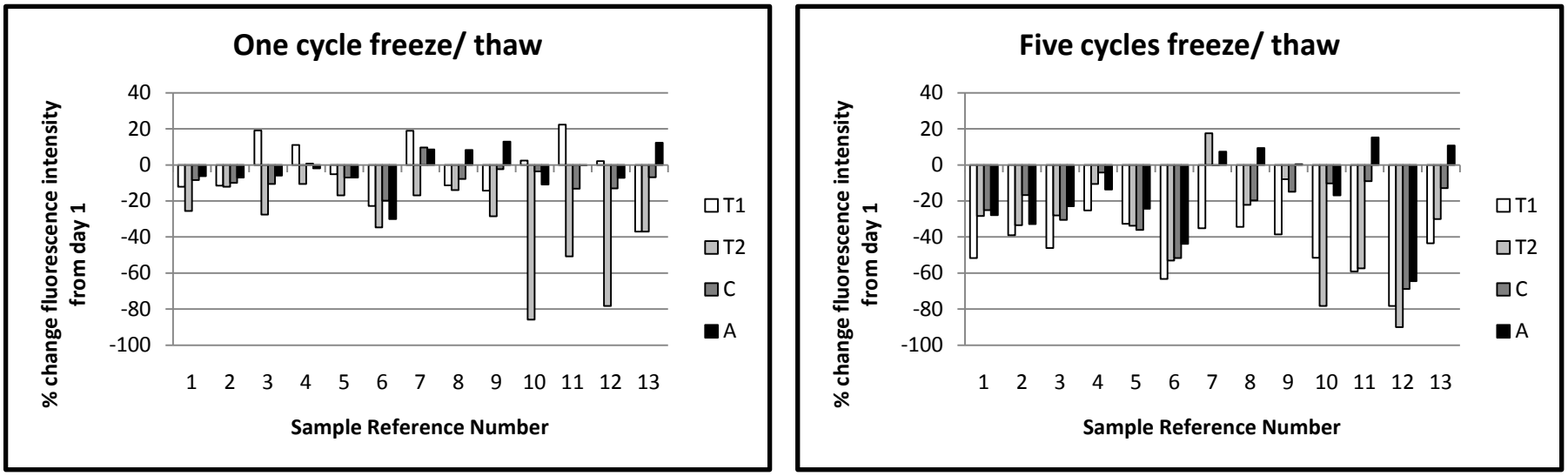
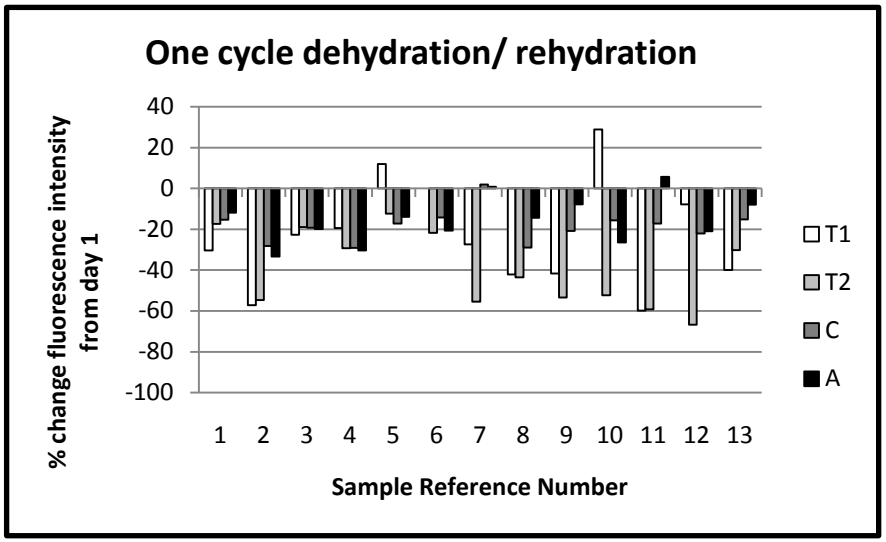


Figure 6.1: Bar chart illustrating differences in fluorescence intensity in peaks T_1 (white), T_2 (light grey), C (dark grey) and A (black) in all samples after one cycle of freezing and thawing and after five cycles of freezing and thawing. A decrease in intensity is apparent for the vast majority of samples.



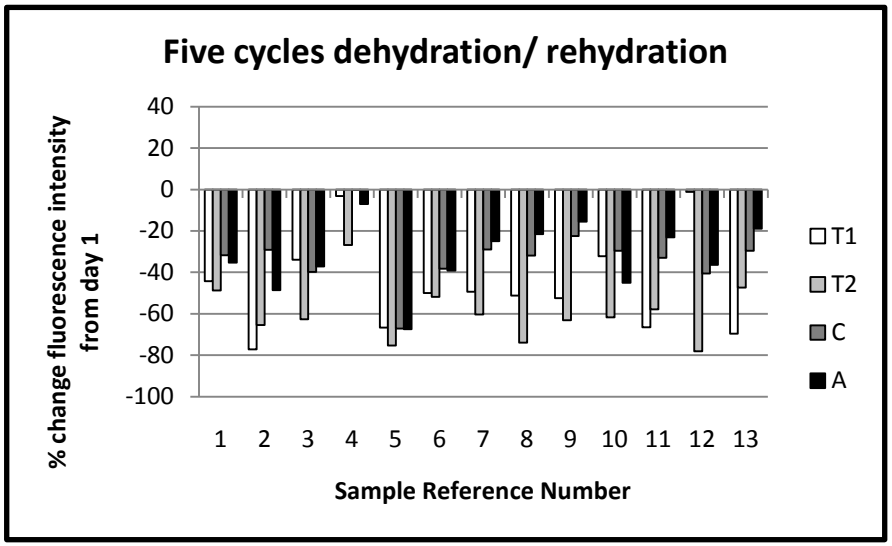
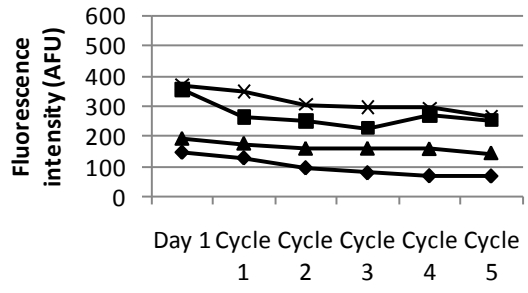
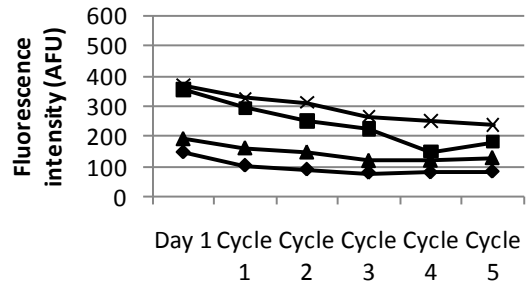
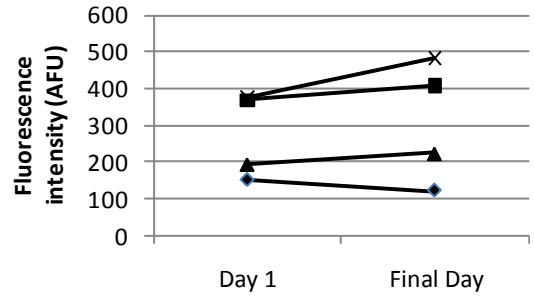
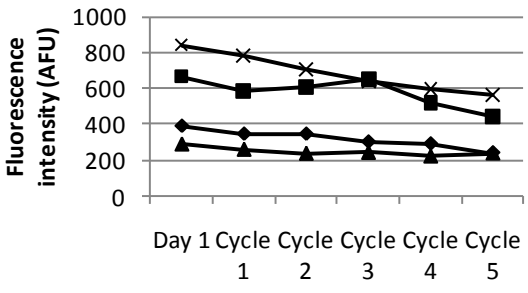
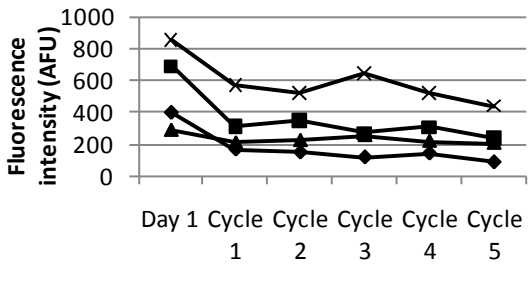
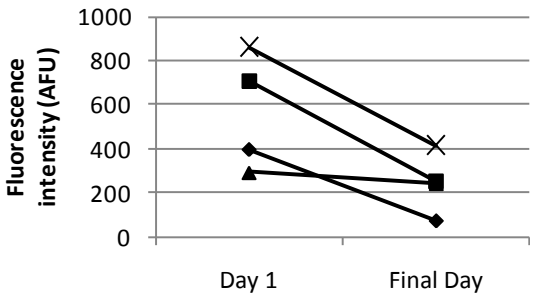
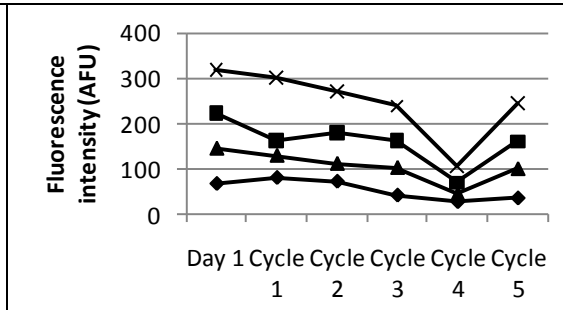
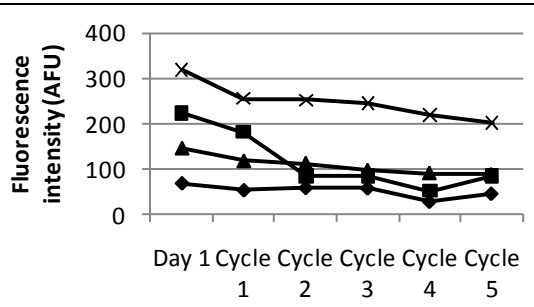
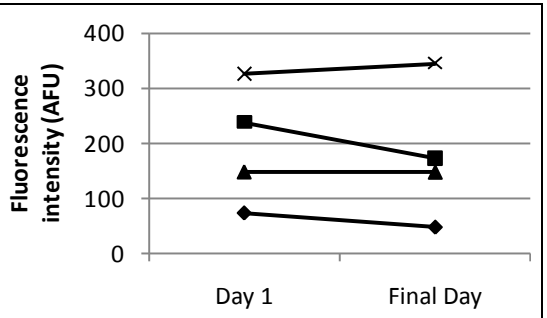
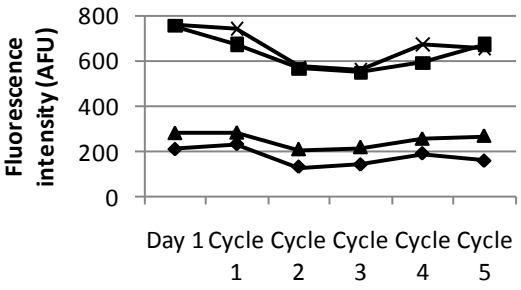
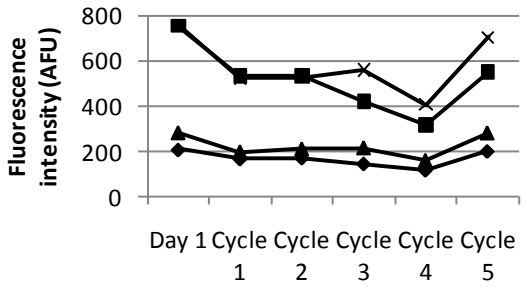
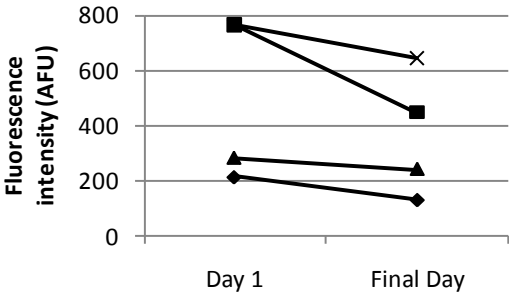
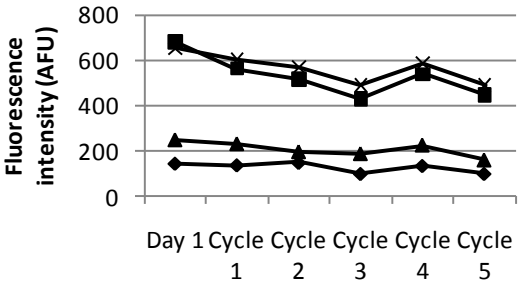
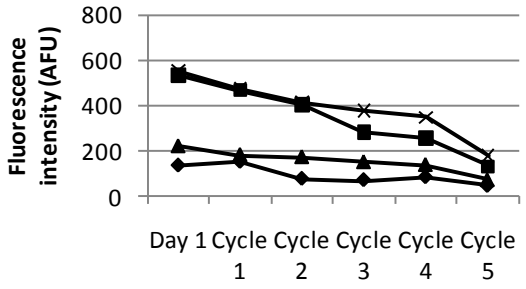
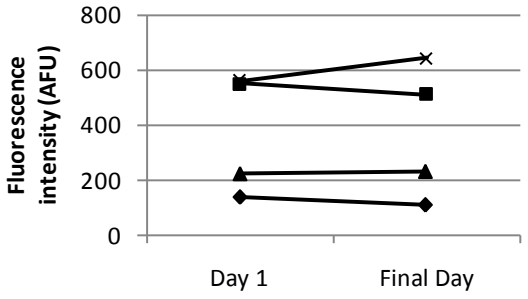


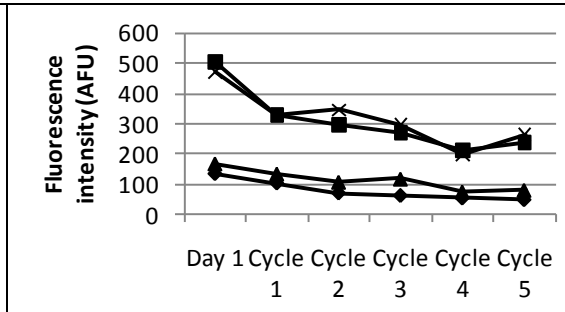
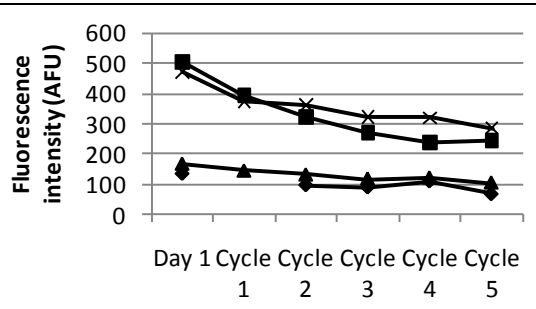
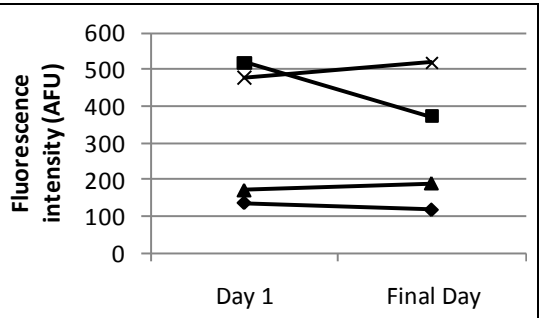
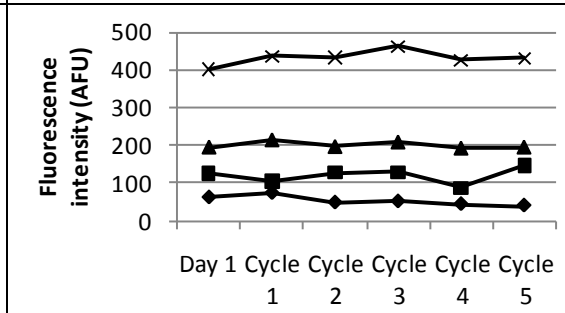
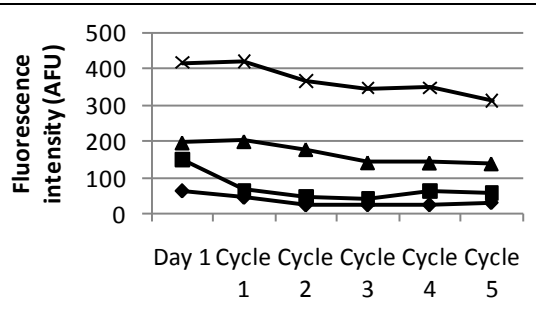
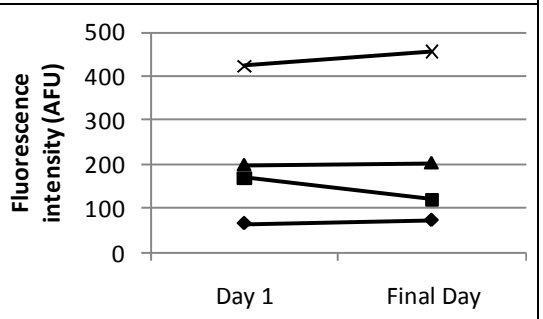
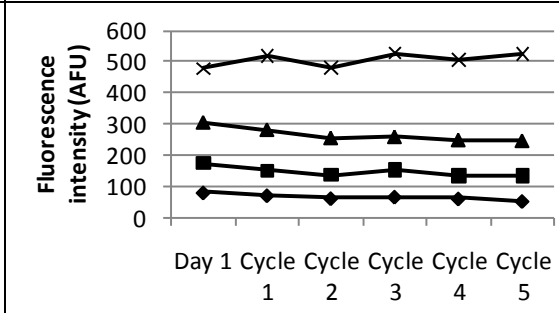
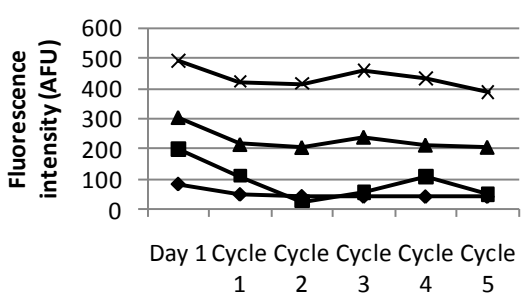
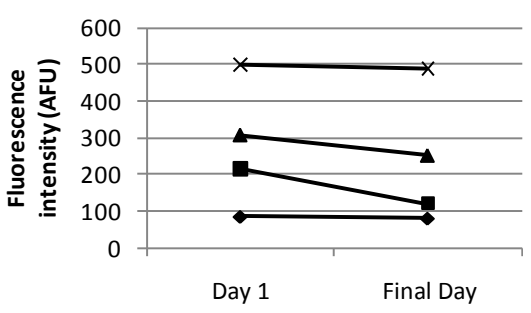
Figure 6.2: Bar chart illustrating differences in fluorescence intensity in peaks T_1 (white), T_2 (light grey), C (dark grey) and A (black) in all samples after one cycle of dehydration/ rehydration and after five cycles of dehydration/ rehydration. A decrease in intensity is apparent for all samples.

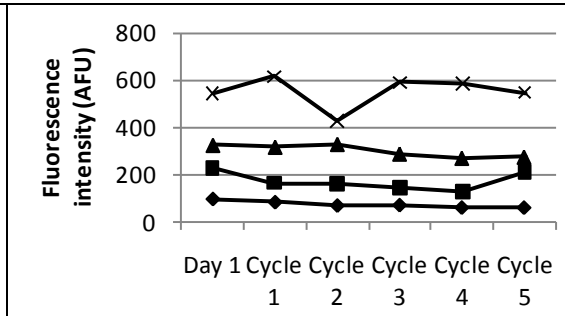
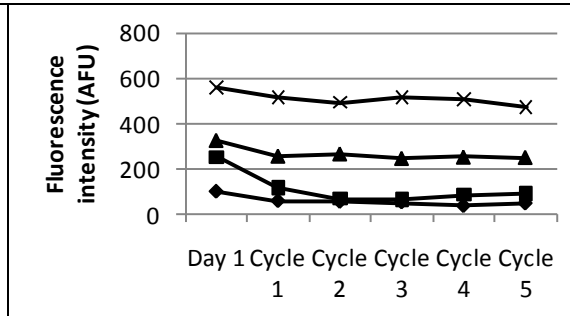
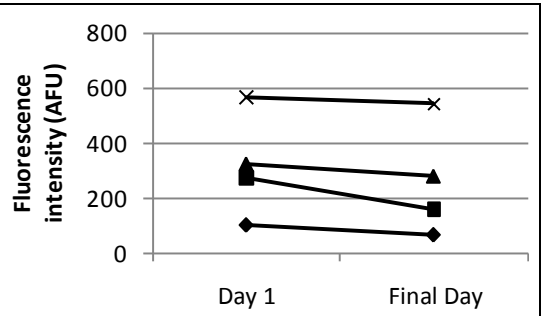
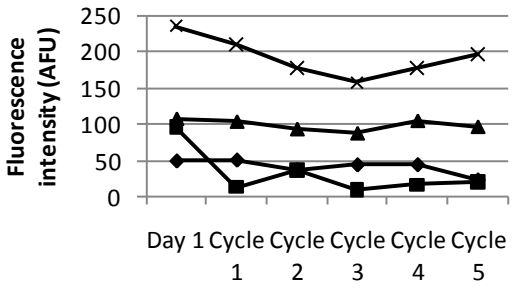
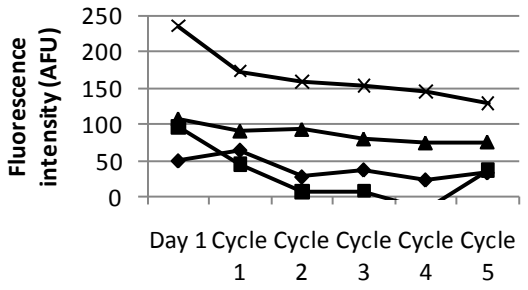
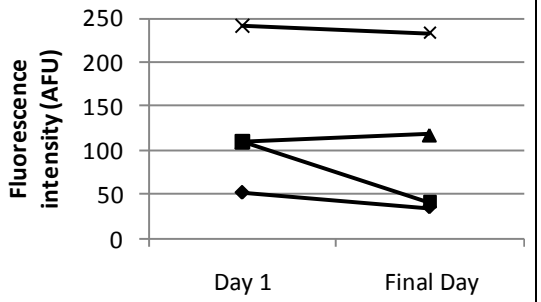
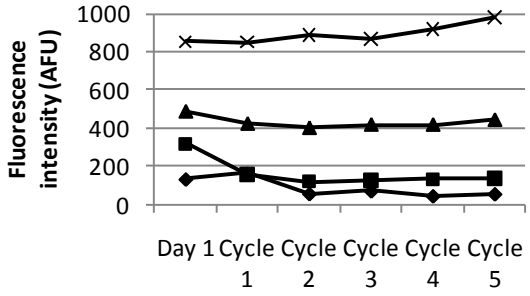
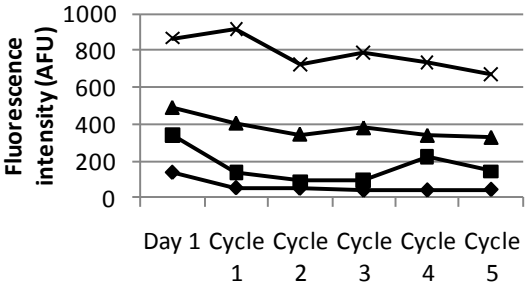
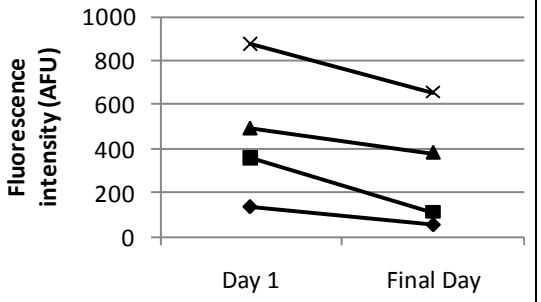
Figure 6.3 illustrates the change in fluorescence intensity for each peak after each cycle of freezing/ thawing, dehydration/ rehydration and the change observed in refrigerated control samples between Day 1 and the final day of analysis. Peak T_1 is identified by a solid line with diamond, peak T_2 by a solid line with square, peak C by a solid line with triangle and peak A by a solid line with cross.

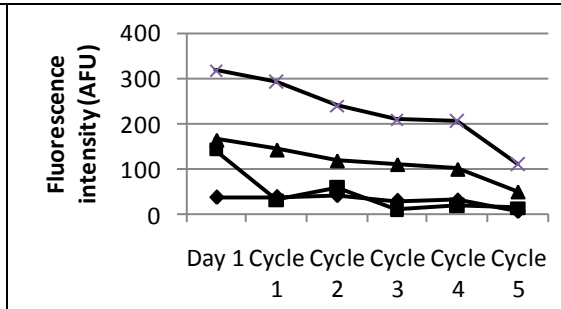
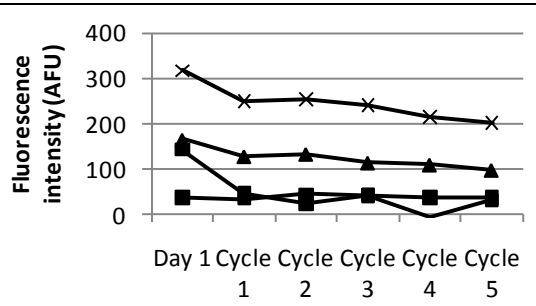
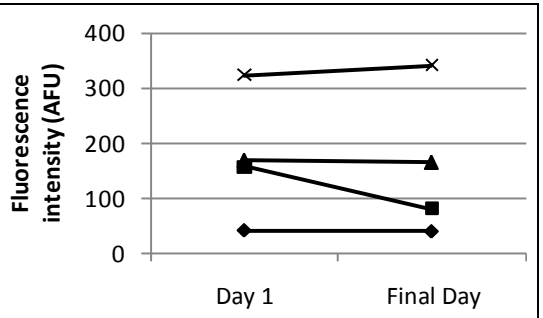
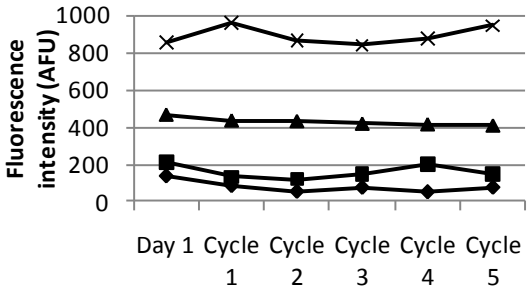
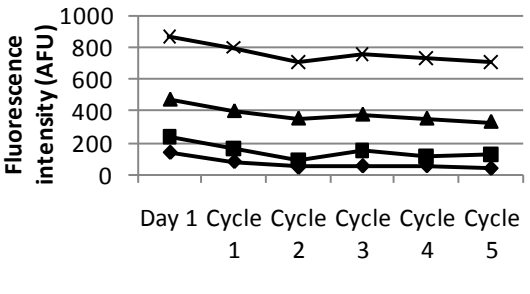
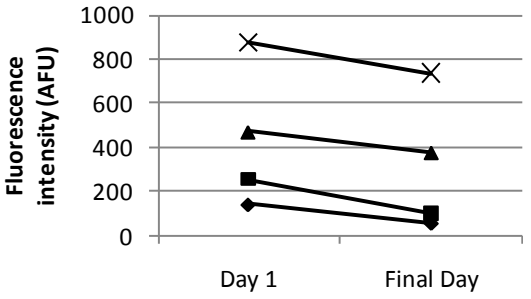
Figure 6.3: The fluorescence intensity of peaks T_1, T_2, C and A as a measured value recorded on Day 1 and after each cycle of freezing/ thawing and dehydration/ rehydration (total five cycles) for each individual sample. Also change in fluorescence intensity in refrigerated control samples between initial analysis and the end of the analysis period. Peak T_1 is identified by a solid line with diamond, peak T_2 by a solid line with square, peak C by a solid line with triangle and peak A by a solid line with cross. In general terms all peaks demonstrate a decrease in fluorescence intensity over repeated cycles of freezing/ thawing and dehydration/ rehydration, a pattern which is not always repeated in the refrigerated control sample.

	Frozen/ thawed	**Dehydrated/ rehydrated**	**Refrigerated control**
River Tame			
Wood Brook			

River Rea			
Harborne Brook North			
Harborne Brook South			

Vale Lake			
Bartley Brook			
Merritt's Brook			

River Trent			
Repton Brook			
Hilton Brook			

River Dove			
Alder Brook			

6.4.5. Changes in Peak Position with Freezing/ Thawing and Dehydration/ Rehydration

To summarise the data the mean changes in fluorescence excitation and emission wavelength for all samples were identified. There is no significant change in excitation or emission wavelength for any peaks as a result of one or five cycles of freezing/ thawing or dehydration/ rehydration.

6.4.6. Changes in Fluorescence Intensity with Freezing/ Thawing and Dehydration/ Rehydration Relative to Original Sample TOC Value

The percentage change in fluorescence intensity for each peak (corrected data) for samples which had undergone one and five cycles of freeze/ thaw and dehydration/ rehydration was correlated with the TOC value recorded on the day of collection. The purpose of this exercise was to determine whether initial organic carbon levels in any way determined the behaviour of the sample under the specific storage conditions. It has previously been stated that high carbon samples are unsuitable for freezing due to instability of the carbon and other nutrients (Spencer et al, 2007; Fellman et al, 2008). In this work it was determined that there is a weak correlation between original TOC and percentage change fluorescence intensity for all peaks for both frozen and dehydrated samples after one cycle. The correlation coefficients (Spearman's rho) are presented in Table 6.9.

Table 6.9: Correlation coefficients (Spearman's Rho) for the relationship between fluorescence intensity (AFU) after one cycle of freeze/ thaw or dehydration/ rehydration and TOC (mgl^{-1}) measured on day of sample collection for all samples (N = 7). All fluorescence data corrected for changes observed in an 18MΏ deionised water control sample subjected to the same conditions of freeze/ thaw or dehydration/ rehydration).

		T_1/ TOC	**T_2/ TOC**	**C/ TOC**	**A/ TOC**
Frozen	Spearman's rho	-0.286	0.821	0.214	0.321
Dehydrated	Spearman's rho	-0.257	-0.786	-0.179	0.000

Table 6.10: Correlation coefficients (Spearman's Rho) for the relationship between fluorescence intensity (AFU) after five cycles of freeze/ thaw or dehydration/ rehydration and TOC (mgl^{-1}) measured on day of sample collection for all samples (N = 7). All fluorescence data corrected for changes observed in an 18MΏ deionised water control sample subjected to the same conditions of freeze/ thaw or dehydration/ rehydration).

		T_1/ TOC	**T_2/ TOC**	**C/ TOC**	**A/ TOC**
Frozen	Spearman's rho	0.429	0.464	0.071	0.071
Dehydrated	Spearman's rho	-0.286	0.393	0.036	0.250

It appears from the data presented for corrected samples above that after one cycle of dehydration/ rehydration there is an inverse correlation (r = -0.786) between the original TOC value and peak T_2 fluorescence intensity. However, there is a stronger positive correlation (r = 0.821) between percentage change T_2 fluorescence intensity and original TOC value for samples which have been frozen and thawed. Peaks T_1, C and A generally display weaker correlations with TOC and it could be suggested that peak T_2 is most strongly influenced by the concentration of organic carbon, while T_1 is influence by different mechanisms and is less impacted. However, this may simply be related to the degree of correction of peak T_2 relative to T_1.

In samples which had undergone five cycles of freezing/ thawing or dehydration/ rehydration the patterns of relationship between fluorescence change and original organic carbon concentration are less clearly defined as illustrated in Table 6.10. Changes in fluorescence intensity for all peaks are generally positively correlated, although in almost all cases the correlation is poor.

It was speculated by Spencer et al,, (2007) and Fellman et al, (2008) that the degree of change in fluorescence and nutrient levels after freezing and thawing respectively may be related to the original character of the water, particularly the original organic carbon concentration. This work demonstrates that the original TOC concentration may indicate likely changes in the labile organic fractions indicated by T_2 fluorescence intensity. However peaks C and A in particular show no clear relationship to original TOC and thus it may be suggested that more stable organic matter represented by these humic-like peaks behaves independently regardless of organic matter concentration and supports the theory that this is a recaltriant fraction even under hostile environmental conditions.

6.4.7. Changes in Fluorescence Intensity with Freezing/ Thawing and Dehydration/ Rehydration Relative to Original Sample Chemical Character

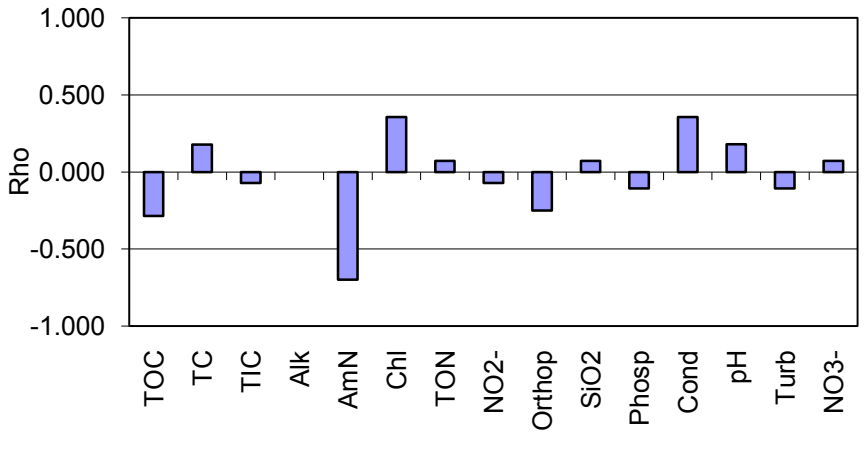
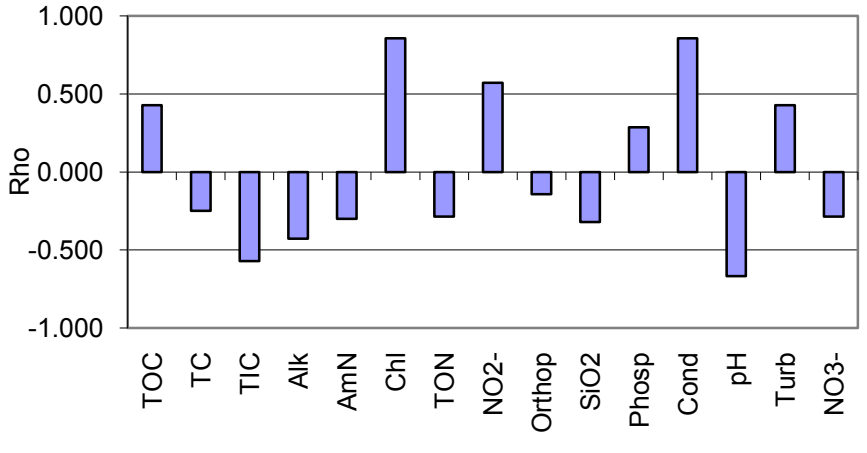
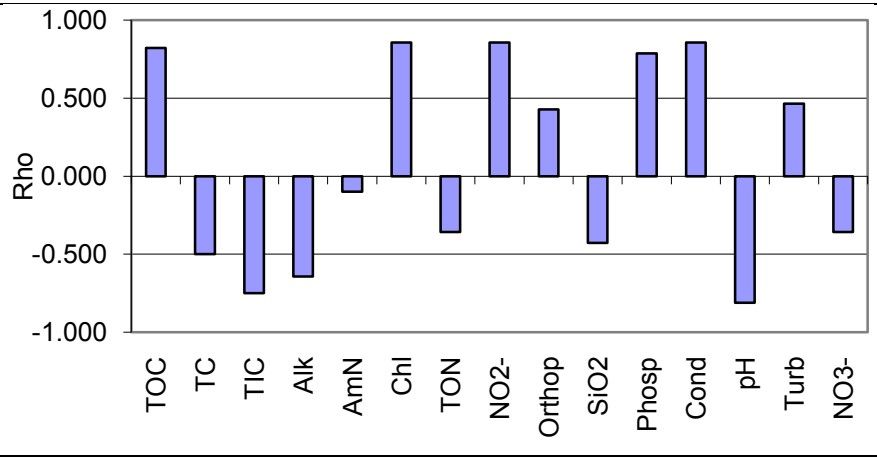
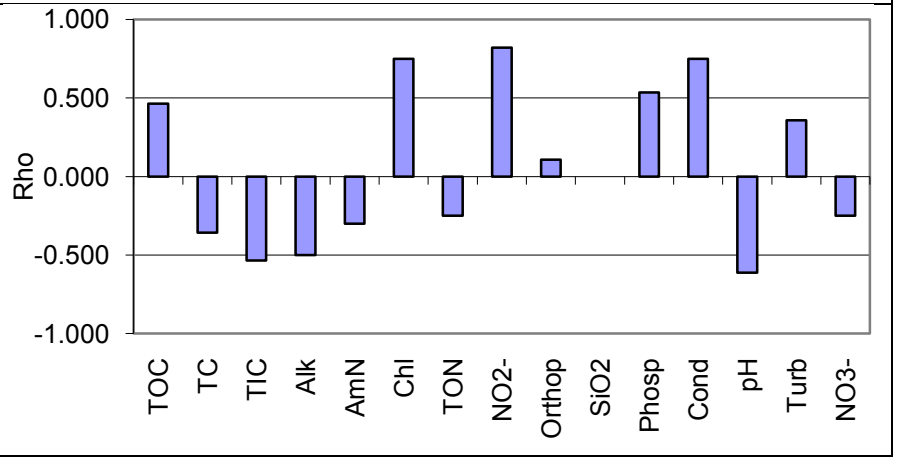
Basic correlations were carried out on the results of the full suite of analyses undertaken by the Environment Agency on the original samples and the percentage changes in fluorescence intensity for each peak after one and five cycles of freeze/ thaw and dehydration/ rehydration. All fluorescence data is corrected as described in 6.4.1.

Due to the relatively "clean" nature of the samples BOD_5 values were often too low for detection at the dilution at which the samples had been analysed. In this case it is not possible to include this parameter in the statistic analysis as only 2 samples returned a numerical value.

Graphs showing the correlation between percentage change fluorescence intensity for each peak and original chemical parameters of the samples after one and five samples of freezing/ thawing and dehydration/ rehydration are presented in Figure 6.4 and 6.5 respectively.

There tend to be positive correlations between change in fluorescence intensity for all peaks and chloride, nitrite and conductivity in addition to those previously discussed between fluorescence intensity of peak T_2 in samples which underwent both one and five cycles of freezing and thawing. This could indicate that the factor that influences degree of fluorescence change is not

actually TOC concentration but the concentration of all ions in solution or that the organic material present is made up of a greater proportion of labile organic matter as these properties are also indicators of wastewater presence or pollution events. Conductivity commonly demonstrates one of the strongest correlations with percentage change fluorescence intensity across all peaks and may potentially be used as an indicator of the degree of change likely to take place in samples during freezing. A far larger dataset would be required to fully determine the practicality and accuracy of this as an indicator or freezing stability.

	One cycle freezing/ thawing	Five cycles freezing/ thawing
T_1		
T_2		

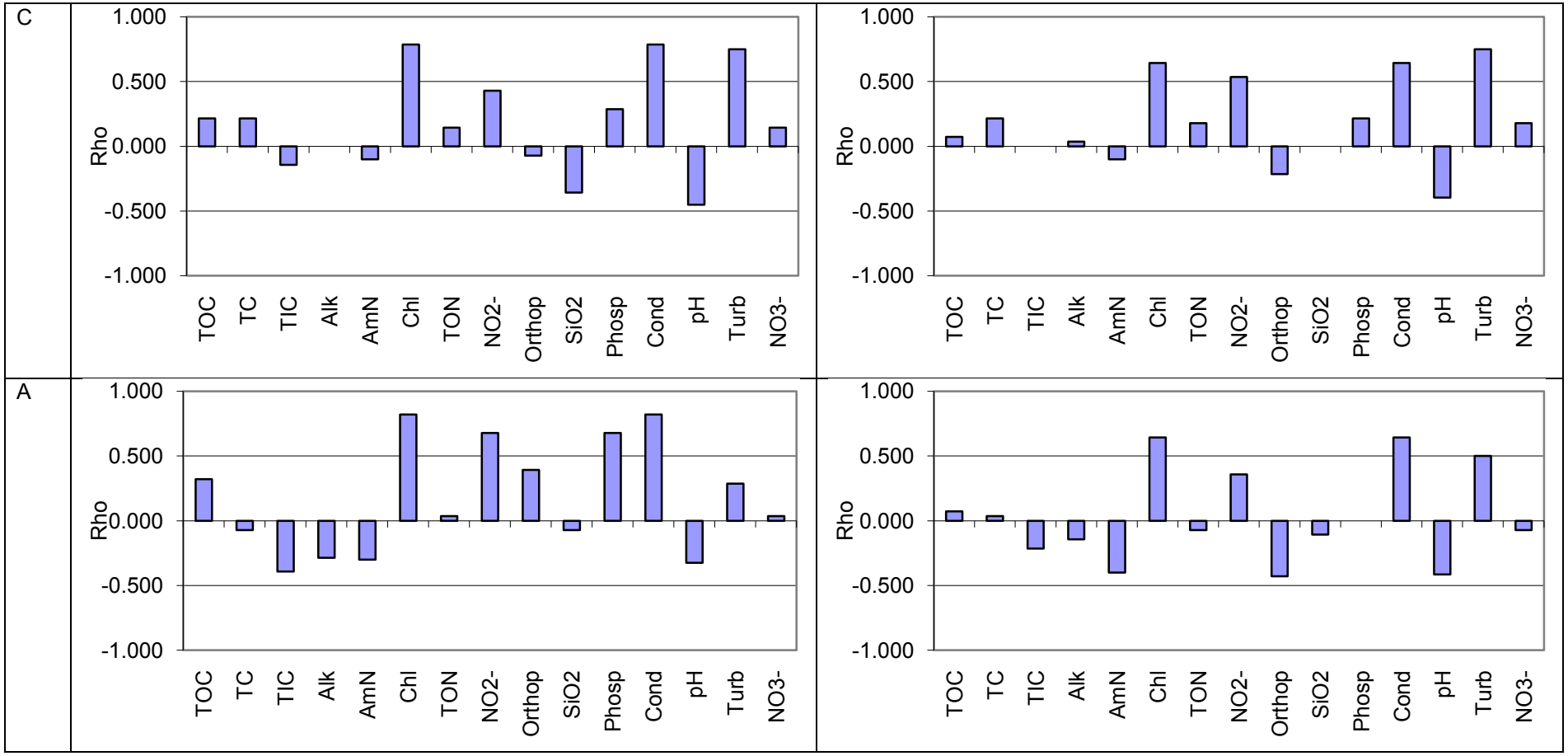
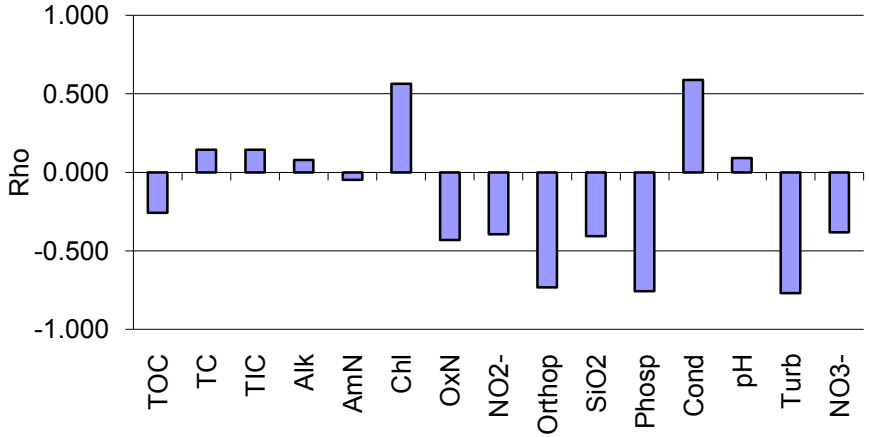
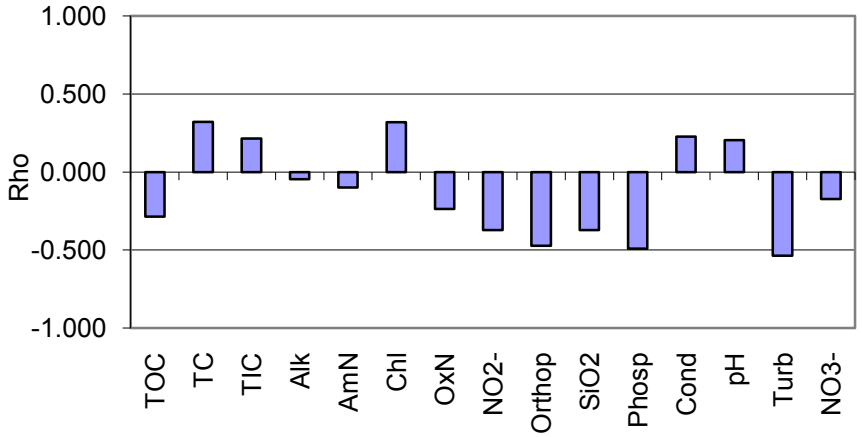
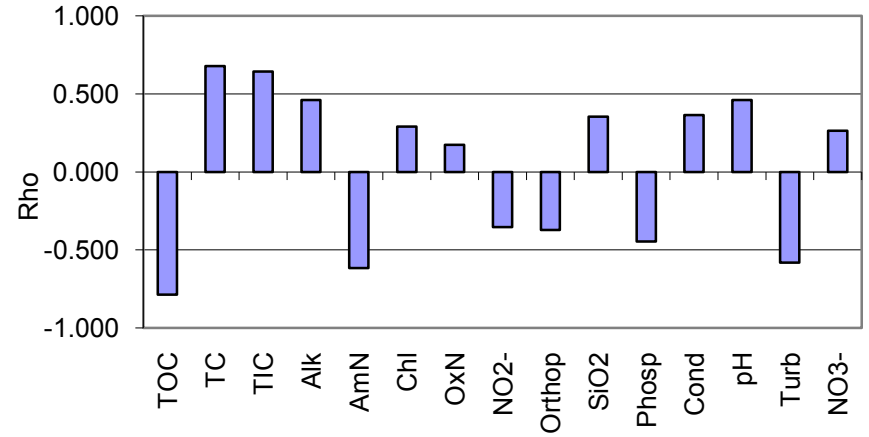
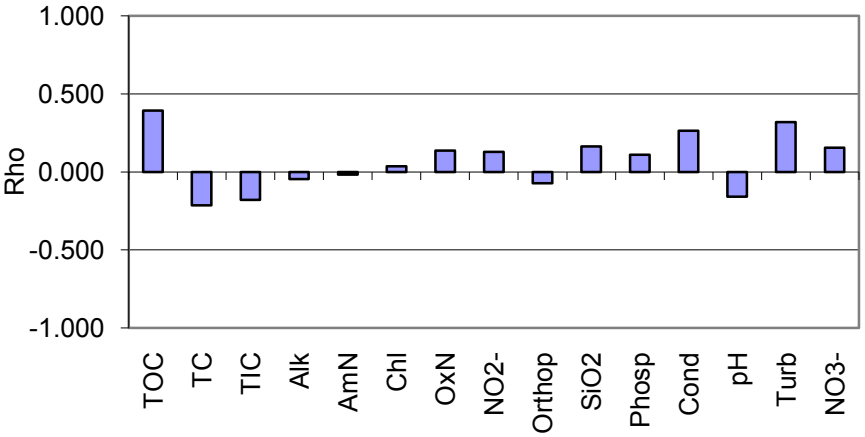


Figure 6.4: Correlation coefficients recorded between fluorescence intensity of peaks T_1, T_2, C and A and water chemistry parameters (TOC, TC, TIC, Alk, AmN, Chl, TON, NO_2^-, Orthop, SiO_2, Phosp, Cond, pH, Turb, NO_3^-) in samples subject to one and five cycles of freezing/ thawing, (all fluorescence data corrected for the response of an 18MΩ deionised water sample subjected to the same conditions of freeze/ thaw).

	One cycle dehydration/ rehydration	Five cycles dehydration/ rehydration
T_1		
T_2		

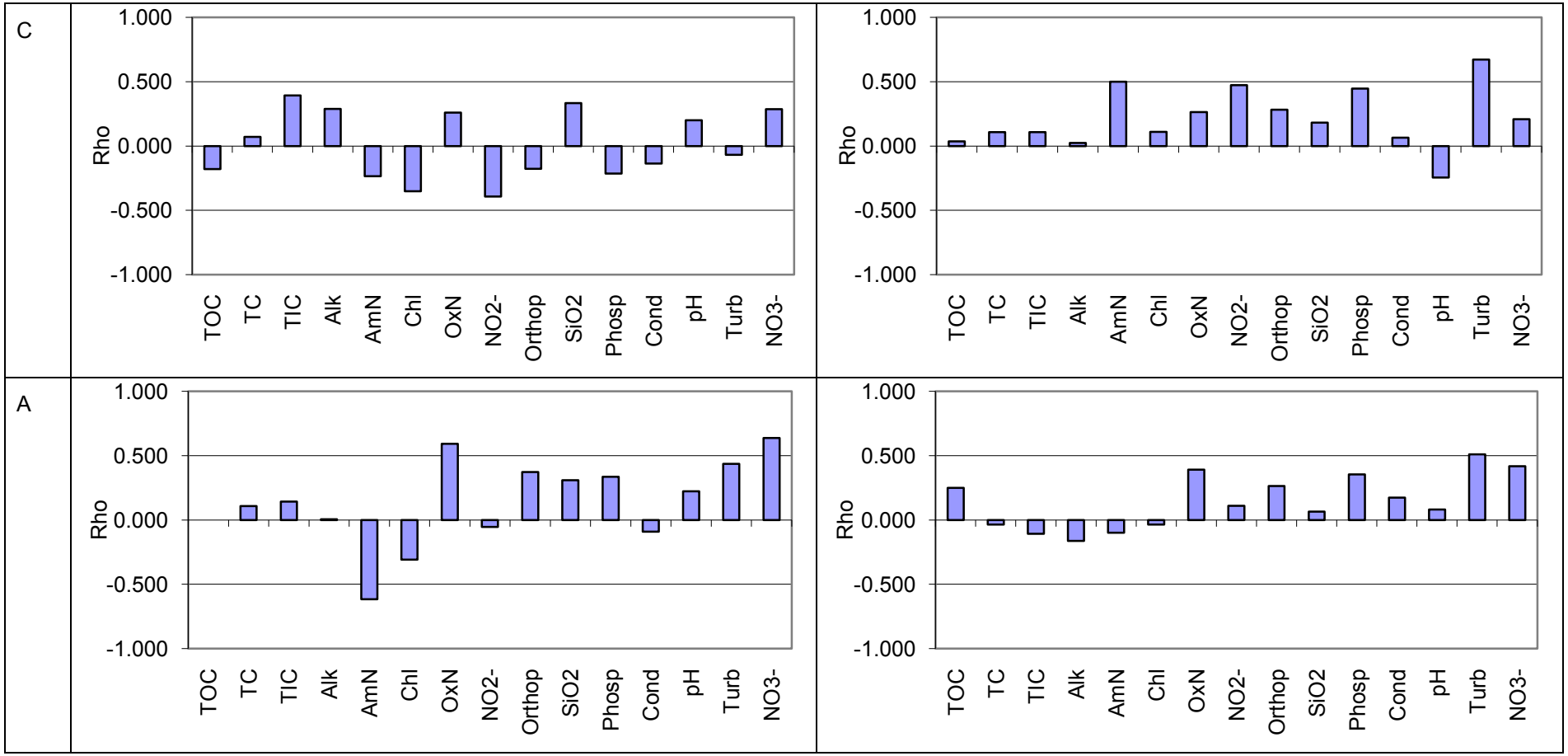


Figure 6.5: Correlation coefficients recorded between fluorescence intensity of peaks T_1, T_2, C and A and water chemistry parameters (TOC, TC, TIC, Alk, AmN, Chl, TON, NO_2^-, Orthop, SiO_2, Phosp, Cond, pH, Turb, NO_3^-) in samples subject to one and five cycles of dehydration/ rehydration, (all fluorescence data corrected for the response of an 18MΏ deionised water sample subjected to the same conditions of dehydration/ rehydration).

Corrected dehydrated/ rehydrated samples do not demonstrate any common relationships between original chemical parameters and the degree of fluorescence intensity change in samples analysed after one or five cycles. This suggests that the effect of the dehydration/ rehydration process on fluorescent organic matter is much more arbitrary and could not be determined by the original character of the sample.

As nutrient levels were only analysed in samples on day one it is not possible to draw any conclusions about relative nutrient stability after freeze/ thaw and dehydration/ rehydration processes in line with the work of Fellman et al., 2008. Furthermore these relationships must be considered against the quality of the correlations. Due to the small sample size (n = 11) even the strongest correlation observed between fluorescence and water quality (r = 0.673 observed between peak C and turbidity in dehydrated/ rehydrated samples after 5 cycles) gives confidence limits of 0.123 to 0.906 at the 95% level.

6.5. Discussion

Chemical water quality results (Table 6.1 and 6.2) are all typical of British freshwaters. Two urban sites, Wood Brook and Harborne Brook North, can be seen to be highly influenced by organic pollution with high BOD_5 values. The urban Vale Lake has high ammonia values with no elevated BOD_5 value, suggesting an autochthonous source of ammonia, rather than an allochthonous source associated with sewage pollution. As all samples fall within that expected for British freshwaters, they have been ordered in this work by

decreasing urban/ increasing rural land cover. Those samples at the more rural end of the spectrum may be seen to be more highly turbid, with higher total oxidised nitrogen and nitrate concentrations, typically of larger rivers and an agricultural source of nitrate pollution. Urban rivers had relatively high chlorine and total organic carbon, indicative of urban runoff and sewerage contamination.

After one cycle of freezing, peaks T_2 and C tend to show changes in fluorescence intensity which fall outside the bounds of analytical uncertainty. This may suggest that these peaks are intrinsically more unstable than the T_1 and A peaks, and suggests that different organic matter fractions contribute to each of the four fluorescence peaks. Few patterns are observed between the change in fluorescence intensity after freezing and chemical water quality, although samples which are classified as more “rural” e.g. Repton Brook, Alder Brook, Hilton Brook and River Trent show large changes in T_2 fluorescence intensity. However, the extent of change in peak T_2 intensity may be influenced by the relatively large correction factor applied to this peak or difficulties identifying the peak which may arise as a result the excitation wavelengths being close to the limit of lamp output. After 5 cycles of freezing the River Dove demonstrates the greatest degree of fluorescence change for all peaks. One sample, Harborne Brook North, demonstrates an increase in fluorescence intensity during later freeze-thaw cycles, suggesting the presence of several organic matter fractions with different sensitivities to freezing.

After one cycle of dehydration/ rehydration most samples generally demonstrate fluorescence decreases greater than analytical uncertainty, particularly in peaks T_2 and C which are greater than those observed in frozen samples. Peak T_1 also decreases significantly. However, as in samples subjected to one cycle of freezing and thawing peaks T_1 and T_2 are seen to change independently of each other. This may suggest separate fluorophores are responsible for fluorescence of these peaks and a greater stability of the T_1 peak. Peak A intensity also decreases with dehydration more than with freezing; this is slightly more apparent for the urban sample sites. One cycle of dehydration therefore causes a greater decrease in fluorescence intensity of all fluorescence peaks that one cycle of freezing. After five cycles of dehydration all fluorescence peaks in all sample sites except the Harborne Brook North exhibit a significant decrease in fluorescence. Harborne Brook North initially decreases in fluorescence, but after five cycles increases in fluorescence for all fluorescence peaks.

There appears to be no simple relationship between the initial sample characteristics and the manner in which the organic matter responds to episodes of freezing/ thawing or dehydration/ rehydration. As a result of a lack of data it is not possible to determine whether there is a relationship between BOD_5 and changes in peak T intensity which would suggest whether the processes determining organic matter degradation with freezing and dehydration are different to those determining microbial degradation. What may be summarised is that there is a common decrease in fluorescence intensity of

all peaks in all samples after freezing/ thawing and dehydration/ rehydration, ubiquitous across all samples, regardless of catchment. Peak C intensity correlates with TOC as demonstrated by Ferrari et al., 2006 and Bieroza et al., 2009; therefore a decrease in peak C intensity of 23% with five freezing cycles and 33% with five dehydration cycles suggests a decrease in TOC of 1-3 mg/l can be inferred. Therefore these, largely unstudied, processes are important in our understanding of carbon cycling in the environment.

6.6. Summary

1. There is a general trend for fluorescence peaks in all samples to decrease in fluorescence intensity after freezing/ thawing and dehydration/ rehydration. This may suggest a decrease in TOC during these processes which is highly important in our understanding of carbon budgets.

2. In general, fluorescence intensity continued to decrease with repeated cycles of freezing and dehydration, although for one urban site, fluorescence increased with repeated cycles.

3. T_1 and T_2 follow independent behaviour in response to freeze/ thaw and dehydration/ rehydration. This may indicate that peaks T_1 and T_2 comprise more than one fluorophore which respond differently to freezing and dehydration.

4. Dehydration and rehydration appears to be more disruptive to organic carbon fluorescence than freezing and thawing, although both are highly destructive and lead to losses of fluorescence after both one and 5 cycles.

5. It is likely that the observed decrease in fluorescence intensity also indicates a loss of TOC from samples through the freezing and dehydration processes. It is not clear from this work whether this is a result of the disruption fluorescent organic molecules, or a result of aggregation and settling of some of the fluorescent moieties.

6. As a result of these findings freezing and/ or dehydration of samples prior to analysis is not recommended due to the poor stability of fluorescent organic matter under conditions of freezing/ thawing and dehydration/ rehydration.

7. In proposing that decreasing fluorescence intensity indicates a decrease in TOC concentration one may speculate that under potential climate change scenarios (Solomon et al., 2007) a reduction in the number and frequency of winter freezing events in mid latitudes, which may occur as a result of global warming, may be lead to an overall increase in TOC concentrations and colour in mid latitude rivers. Conversely, in high latitudes, defrosting and freezing due to permafrost melting might be increasing, leading to an increase in TOC in rivers. At lower latitudes,

dehydration events may occur more frequently as a result of global warming, which could contribute to a decrease in TOC concentrations in rivers in this region.

7. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

The aim of this PhD. was to investigate organic matter fluorescence in some fresh and waste waters with an emphasis on using fluorescence to indicate the character and dynamics of organic carbon in the environment. To do this I undertook a study of the labile or recaltriant character of the organic matter with a view to understanding the oxygen depleting load of organic carbon in water and the potential effects on the aquatic ecosystem. This work was undertaken on a range of river waters and sewage effluents to characterise the organic load of waters from different sources by analysis of the fluorescence of common peaks known to be more or less bioavailable. I investigated the possibility of using fluorescence as a tool to indicate water quality by relating fluorescence characters to common measures of water quality to see whether fluorescence could be used as an indicator of polluting potential of individual waters based on the concentration and character of organic carbon.

Furthermore, I investigated the effect of a range of environmental conditions upon the character, concentration and structure of organic carbon in surface waters indicated by changes in fluorescent signature. I identified whether the initial character of the organic carbon influences the potential change in character and concentration and suggested ways in which changes in fluorescence may indicate changes in molecular structure.

This work has relevant industrial applications. I report on the potential to use fluorescence spectroscopy as an on-site and laboratory indicator of organic pollution in water, a low cost, rapid analytical method to replace existing techniques which are slow and in some instances, costly. It is also of hydrological interest as it presents some novel results regarding the dynamics of organic carbon in water under a range of environmental conditions. These are relevant to analytical protocol, as they indicate that certain methods of samples storage should lead to low confidence in analytical results. They are also relevant to the understanding of the impact of changing climate. An increase in freezing or dehydration events at different latitudes (Solomon et al., 2007) may ultimately lead to decreased or increased TOC concentrations in rivers impacting upon the balance of carbon processing and transport within rivers.

The major fluorophores identified in the surface waters and sewage effluents studied were protein-like peak tryptophan, represented as a double peak (T_1 and T_2) and humic-like peaks C and A. Peaks T_1 and T_2 were determined to be labile, correlating well with BOD_5 a measure of biodegradable organic matter, although models indicated that T_1 is a poor proxy for the BOD_5 test. Peaks C and A were determined to be less labile than peaks T_1 and T_2 (demonstrating weaker correlations with BOD_5) however they were not found to be recaltriant, and were influenced by a range of environmental conditions.

While peaks T_1 and T_2 both correlated well with BOD_5 they displayed other features which indicated that they may have different origins, structures or may occur in different positions on protein molecules – being more or less exposed to environmental influence. Peaks T_1 and T_2 were found to decrease with increasing temperatures when stored between 4°C and 20°C in the dark, but increased in intensity when stored in light/ dark cycles. Peak T_2 fluorescence tended to change to a greater degree than T_1 after cycles of freezing/ thawing and dehydration/ rehydration. These findings suggest that the T_2 fluorophore may be positioned in a more exposed location within organic carbon molecules, making it more susceptible to environmental influence in the fluorescence process. It may also be speculated that peak T_2 is less susceptible to pH change in solution. This is indicated by smaller changes in T_2 fluorescence than in T_1 fluorescence in samples in which pH change is observed. It is feasible that this may indicate uncoiling or extension of the molecules with increasing alkalinity (Myneni et al., 1999), exposing previously protected T_1 fluorophores and exposing them to environmental influences and light applied for fluorescence analysis. However, this may also indicate a breakdown of larger molecules to smaller more bioavailable photo-products supported by a blue-shift in both T_1 and T_2 emission wavelengths, as pH change is only observed in samples exposed to light.

It should, however, be mentioned that changes in peak T_2 intensity may be simply reflecting interference from the highly fluorescence neighbouring peak A affecting identification of the peak. Furthermore, peak T_2 is excited at

wavelengths which are close to the limit of the Cary-Eclipse lamp. At these wavelengths the lamp output may be more prone to variation, affecting the recorded shape of the T_2 peak.

Peaks C and A in surface waters and effluents are not highly susceptible to microbial degradation as they did not correlate strongly with measures of bioavailability. The humic-like peaks did correlate well with TOC and COD suggesting that humic material constitutes the greatest volume of and may be an indicator of total organic carbon concentration in surface waters. However, in effluents, peak T_1 demonstrated a similar correlation with TOC to peak C suggesting that the proportions of bioavailable and less bioavailable organic carbon in sewage effluents are more similar and that humic-like fluorescence should not be used as an indicator of total organic carbon concentration in these waters. Peak C correlated more strongly with COD in surface waters suggesting that this is a less bioavailable fraction derived from terrestrial plant breakdown. However, peak A also correlated strongly with COD in surface waters and effluents which suggests that there is an element of less bioavailable microbial derived humic-like material present. This is supported by an increase in peak C and A fluorescence which corresponds with significant CFU counts in samples stored at higher temperatures in the dark. Peak C and A fluorescence are also enhanced in samples in which pH change is observed. Avena et al., (1999) suggest that this would be a result of molecular shrinkage, but it may also be speculated that, in this case, it is a result of the photo-production of smaller molecules.

In these surface waters all fluorophores are present in the dissolved fraction. However, this is contrary to findings by other authors working on similar waters who identified most of the peak T fluorescence in the particulate fraction (Lead et al., 2006; Baker et al., 2007). Change in peak T fluorescence, in surface waters, was often found to be higher in the dissolved fraction (particularly in samples exposed to light during storage) than that in the colloidal/ dissolved fraction and occasionally the unfiltered fraction. This is an indication that some degree of cell damage is likely to have occurred during the process of vacuum filtration, causing leakage of cell contents rich in tryptophan-like fluorescence. It may also indicate production of small tryptophan-like rich molecules by algae, growing in light conditions. This may be the reason for the deviation from the previous findings in which samples tend to be stored under standard conditions of cold and dark.

Tryptophan-like fluorescence was found to be a good indicator of bioavailable organic matter in both surface waters and effluents, which have markedly different fluorescence and organic carbon concentration characters. It also correlated well with ammonia suggesting that fluorescence may, in fact, be a rather blunt but effective tool for identifying the presence of pollution incidents in surface waters. To maximise the potential of the fluorescence technique in water quality monitoring it is necessary to “uncouple” it from BOD_5 and not use it as a simple proxy for the existing technique. Fluorescence spectroscopy could be a far more powerful tool for characterising waters, rapidly reflecting the

proportions of more and less biodegradable fractions present. However, to strengthen the application of the technique the tryptophan/ BOD_5 relationship should be investigated over a longer term for individual sites to determine whether it is possible to develop a specific site-by-site relationship with excellent correlations and regression models between the two parameters. Application of the technique, under the constraints of current understanding, is limited by the heterogeneity shown by the wide ranging samples studied although it does work as a general indicator of pollution. Compilation of such site specific data may allow a better understanding of the general character of organic matter in certain types of environments, enabling pollution events, or deviations from the normal condition to be clearly identified.

There were generally poor correlations between fluorescence and inorganic measures of “nutrient” concentrations – nitrate and orthophosphate. It appears that in the waters analysed nitrate and phosphate have no association with organic carbon and so may not even indicate a shared origin e.g. wash off from agricultural land.

The organic carbon present in the surface waters analysed was strongly affected by environmental conditions and was found to be unstable under almost all conditions, including those that are the current standards for samples storage. In general terms in samples stored in the dark TOC concentration increased regardless of temperature. This was observed in association with an increase in humic-like fluorescence. In samples exposed to light the TOC

concentration decreased most likely through consumption during algal photosynthesis. Humic-like fluorescence remained relatively constant being relatively non-bioavailable, and was perhaps slightly augmented by dead cell material over time. Tryptophan-like intensity increased probably as a result of the release of protein-rich algal products exceeding the uptake of labile material.

Furthermore, surface water organic matter was found to be highly unstable in freezing/ thawing and dehydration/ rehydration conditions. In all instances the fluorescence intensity decreased indicating a loss of organic carbon. There was little clear relationship between original TOC concentration or other water chemistry parameters and the degree of fluorescence change, although conductivity tended to show strong relationships in samples subject to freezing/ thawing perhaps indicating that the ionic make up of the solution was an influencing factor in the degree of fluorescence change.

Surface water organic matter is inherently unstable under all storage conditions, but particularly in the presence of light where photosynthesis is not inhibited. The patterns of fluorescence change were variable and so it is not possible to predict an overall degree of fluorescence change that is likely to occur while a sample is being stored. Even “filter sterilization” does not render the sample inert. Despite this uncertainty recommended storage conditions for water samples would be at 4°C in the dark with a baseline fluorescence measurement taken immediately after sample collection for reference.

APPENDIX 1

Chemical Water Quality Data

2003-2005	River Name	Description	BOD AVE	BOD SD	BOD 90%ile	Grade	Amm AVE	Amm SD	Amm 90%ile	Grade	DO AVE	DO SD	DO 10%ile	Grade	U/S X	U/S Y	D/S X	D/S Y
B	Repton Brook	TRIB FROM HARTSHORN TO RD BR REPTON	1.4	0.98	2.58	B	0.039	0.034	0.077	A	96.12	8.11	85.72	A	431200	321700	430700	326600
A	River Dove	R. TEAN TO FOSTON BK	1.6	0.63	2.43	A	0.052	0.049	0.106	A	96.58	7.08	87.5	A	410600	334400	419480	329870
C	Bourne Brook	DITCH FROM CALIFORNIA TO CONF. R. REA	2.44	1.36	4.15	C	0.111	0.081	0.208	A	93.77	8.67	82.65	A	401000	283300	406300	283540
C	River Rea	NORTHFIELD TO B4217 CANNON HILL	2.64	1.24	4.24	C	0.121	0.081	0.22	A	92.63	5.09	86.11	A	402600	278900	406800	284100
D	River Tame	R REA TO WATER ORTON BRIDGE	3.57	2.28	6.37	D	0.443	0.514	0.945	C	82.05	16.4	61.06	C	410700	289700	41740	291410
A	Distilled Deionised water																	

Water Framework Directive Characteristics

River Name	WFD River I.D	Risk Category	Heavily modified?	River Basin	Catchment size	Point source pollution?	Diffuse pollution?	Water abstraction and flow control?	Physical/ morphological alteration?	Alien species
Repton Brook	GB104028047390	At risk	No	Humber	23.93km2	Not at risk	At risk	Not at risk	Probably not at risk	Probably not at risk
River Dove	GB104028052420	At risk	No	Humber	69.93km2	At risk	At risk	Probably not at risk	Probably not at risk	Probably not at risk
Bourne Brook	GB104028042540	At risk	Provisional	Humber	27.91km2	Not at risk	At risk	Not at risk	Probably not at risk	Probably not at risk
River Rea	GB104028042550	At risk	Provisional	Humber	16.23km2	Not at risk	At risk	Probably not at risk	At risk	Probably not at risk
River Tame	GB104028046840	At risk	Provisional	Humber	90.57km2	At risk	At risk	Probably not at risk	At risk	Probably not at risk
Distilled Deionised water										

APPENDIX 2

				Fluorescence Intensity (AFU)					mgL^{-1}					
Sample Name	SW/ Eff	Date of sampling	hrs from collection	T1	T2	C	A	SMF-2	TOC	AmN	BOD	COD	NO3-	Orthop
685629	SW	14/03/2005	48	21	61	63	127			<0.03	1.13		5.21	0.103
685668	SW	14/03/2005	48	53	119	99	195			0.414	3		4.87	0.078
685669	SW	14/03/2005	72	19	71	25	61			<0.03	1.15		5.99	<0.02
685670	SW	14/03/2005	48	23	65	21	56			<0.03	1.36		5.97	<0.02
685672	SW	14/03/2005	72	88	195	131	223			1.61	3.11		9.27	1.61
685674	SW	14/03/2005	72	27	97	47	90			0.033	1.15		4.2	0.187
685675	SW	14/03/2005	48	17	78	39	84			0.067	<1		4.65	0.162
685676	SW	14/03/2005	48	13	48	26	62			<0.03	<1		5.21	<0.02
685687	SW	14/03/2005	48	12	39	19	47			<0.03	<1		7.48	<0.02
685689	SW	14/03/2005	48	12	28	23	60			<0.03	<1		6.11	<0.02
685690	SW	14/03/2005	48	16	62	47	105			0.159	<1		2.3	0.028
685691	SW	14/03/2005	48	42	87	57	102			0.134	2.12		6.32	0.2
685692	SW	14/03/2005	72	56	105	70	119			0.149	2.5		6.49	0.306
685693	SW	14/03/2005	48	41	79	59	95			0.135	2.05		6.3	0.152
685706	SW	14/03/2005	72	66	141	130	243			0.067	3.56		3.15	<0.02
685707	SW	14/03/2005	48	30	71	153	286			0.035	1.29			
685708	SW	14/03/2005	72	37	84	78	172			<0.03	1.82		5.06	<0.02
685709	SW	14/03/2005	48	16	52	75	178			<0.03	1.2		2.38	<0.02
685722	SW	14/03/2005	48	17	43	43	79			<0.03	1.44		5.29	0.055
685723	SW	14/03/2005	48	15	47	48	93			<0.03	1		5.63	0.042
685724	SW	14/03/2005	48	14	50	40	90			<0.03	<1		5.51	0.044
685725	SW	14/03/2005	48	32	87	54	99			0.063	1.58		7.46	0.115
685726	SW	14/03/2005	72	13	40	33	56			<0.03	1.01		9.64	<0.02
685727	SW	14/03/2005	48	13	40	33	54			<0.03	1.13		6.73	<0.02
685728	SW	14/03/2005	72	36	67	40	92			0.044	1.98		6.77	0.038
685729	SW	14/03/2005	48	12	26	30	59			<0.03	1.12		8.47	0.065
685730	SW	14/03/2005	72	16	40	35	74			<0.03	1.1		8.74	0.022
685731	SW	14/03/2005	72	23	50	41	85			<0.03	1.2		5.66	0.0424
685848	Eff	14/03/2005	72	322	446	340	390			8.66	10.6			
685865	Eff	14/03/2005	48	828	2417	414	752			20.5	22.5		0.55	2.12
694516	SW	11/04/2005	72	14	56	25	66	24		<0.03	1.3		3.44	0.024
694517	SW	11/04/2005	72	10	54	26	65	17		<0.03	1.19		2.66	0.026
694518	SW	11/04/2005	72	18	59	41	95	33	1.75	<0.03	1.44		5.01	0.058
694519	SW	11/04/2005	72	25	63	20	57	27	-0.92	<0.03	2.71		5.17	0.064
694520	SW	11/04/2005	72	44	149	102	191	72	3.46	<0.03	<1		6.41	1.4
694521	SW	11/04/2005	72	31	120	14	161	54		0.033	<1		4.64	0.035
694522	SW	11/04/2005	72	19	59	50	96	33		<0.03	<1		10.4	<0.02
694523	SW	11/04/2005	72	327	365	399	837	249		1.24	11.1			

694524	SW	11/04/2005	72	18	91	77	153	47		<0.03	1.01		7.36	0.044
694525	SW	11/04/2005	72	19	57	68	132	42		<0.03	<1		8.68	0.026
694526	SW	11/04/2005	72	26	98	70	132	49	1.01	<0.03	1.01		8.53	0.048
694527	SW	11/04/2005	72	23	90	54	123	40	1.81	<0.03	<1		6.38	0.123
694528	SW	11/04/2005	72	23	85	59	120	45		<0.03	<1		7.56	0.032
694529	SW	11/04/2005	72	23	93	57	124	47	1.42	<0.03	1.05		7.85	0.09
694530	SW	11/04/2005	72	33	121	66	136	50	1.83	<0.03	<1		7.65	0.079
694541	SW	11/04/2005	72	46	114	109	201	75	4.95	0.113	1.26		9.85	1.05
694542	SW	11/04/2005	72	65	127	163	286	90	5.70	0.095	1.73		8.87	0.161
694543	SW	11/04/2005	72	32	98	97	208	64	5.00	<0.03	<1		3.46	0.145
694545	SW	11/04/2005	72	21	73	55	112	36		<0.03	2.13		5.94	0.059
694546	SW	11/04/2005	72	10	30	25	65	20		<0.03	<1		6.41	0.022
694547	SW	11/04/2005	72	15	54	29	83	29	0.51	<0.03	1.88		6.29	0.027
694548	SW	11/04/2005	72	59	124	206	393	117	6.89	<0.03	1.65		4.97	0.133
694549	SW	11/04/2005	72	22	78	55	112	43		0.211	1.33		5.98	0.069
694550	SW	11/04/2005	72	53	151	155	307	98	6.75	0.088	1.81		5.15	0.196
694581	Eff	10/04/2005	96	257	314	286	345		16.65	3.88	16.4			
694582	Eff	10/04/2005	96	371	302	277	287		15.08	2.74	16.1			
694583	Eff	10/04/2005	96	163	215	208	275		10.46	<0.5	5.96			
694586	Eff	10/04/2005	96	289	934	421	526		31.55	3.84	7.94			
694594	Eff	11/04/2005	72	245	699	227	435		10.73	16.8	11.5			
694606	Eff	11/04/2005	72	216	589	271	486		13.16	16.9	3.22			
694631	Eff	11/04/2005	72	162	232	212	254		5.65	<0.5	<2.92			
694637	Eff	11/04/2005	72	236	396	244	303		12.65	1.67	16			
694663	Eff	11/04/2005	72	203	358	233	326		16.07	<0.5	5.88			
694664	Eff	11/04/2005	72	233	348	235	308		13.13	1.66	8.56			
694666	Eff	11/04/2005	72	205	234	407	445		16.78	3	5.18			
694668	Eff	11/04/2005	72	234	485	148	254		11.97	<0.5	16.9			
694669	Eff	11/04/2005	72	266	320	301	359		8.31	2.38	13.9			
694670	Eff	11/04/2005	72	247	326	257	319		12.53	0.92	15			
694673	Eff	11/04/2005	72	147	225	200	270		7.14	0.65	<2.92			
694674	Eff	11/04/2005	72	289	408	312	420		16.42	10	23.2			
694677	Eff	11/04/2005	72	153	269	207	302		8.87	<0.5	3.31			
694678	Eff	11/04/2005	72	124	289	195	288		20.24	<0.5	<2.92			
694680	Eff	11/04/2005	72	60	175	69	175		3.49	<0.5	<2.92			
694682	Eff	11/04/2005	72	234	326	295	363		15.35	<0.5	9.06			
694698	Eff	11/04/2005	72	509	831	388	591		30.59	<0.5	13.3			
701213	SW	29/04/2005	120	35	109	84	230	85	2.54	<0.03	<1		2.07	<0.02
701214	SW	29/04/2005	120	59	119	218	362	119	12.32	0.057	1.17		7.28	0.35
701216	SW	29/04/2005	120	47	78	149	279	87	4.21	<0.03	1.54		6.94	0.036
701218	SW	29/04/2005	120	18	65	47	106	30	1.74	<0.03	<1		1.2	<0.02
701219	SW	29/04/2005	120	27	66	83	104	52	1.50	<0.03	<1		0.556	0.025
701220	SW	29/04/2005	120	18	43	51	85	33	1.54	<0.03	<1		1.93	<0.02

701221	SW	29/04/2005	120	23	52	48	87	36	1.94	<0.03	<1		7.13	0.058
701224	SW	29/04/2005	120	20	61	87	171	45	2.66	<0.03	<1		4.79	0.042
701225	SW	29/04/2005	120	16	55	73	169	38	3.05	<0.03	<1		2.25	<0.02
701226	SW	29/04/2005	120	17	44	47	110	30	2.52	<0.03	<1		3.26	<0.02
701233	SW	29/04/2005	120	13	42	31	70	23	1.34	<0.03	<1		4.91	0.029
701234	SW	29/04/2005	120	13	43	55	90	33	-0.05	0.034	<1		4.87	0.032
701235	SW	29/04/2005	120	17	55	55	105	32	2.16	<0.03	<1		3.84	<0.02
701271	SW	29/04/2005	120	21	41	113	229	55	5.49	<0.03	<1		3.63	0.034
701272	SW	29/04/2005	120	14	90	55	140	30	3.14	<0.03	<1	<12	0.496	<0.02
701273	SW	29/04/2005	120	24	88	83	180	39	3.58	<0.03	<1	<12	0.216	<0.02
701274	SW	29/04/2005	120	23	97	80	177	38	3.65	<0.03	<1	12	0.326	<0.02
701295	SW	29/04/2005	120	15	45	59	124	33	2.58	0.046	1.08		2.84	0.095
701307	SW	29/04/2005	120	15	43	57	122	31	2.35	<0.03	1.08		2.5	<0.02
701312	Eff	29/04/2005	120	19	52	60	122	33	3.09	<0.03	<1			
701313	Eff	29/04/2005	120	15	51	57	132	33	2.65	<0.03	<1			
701314	Eff	29/04/2005	120	16	57	56	132	36		<0.03	<2.92			
701315	SW	29/04/2005	120	15	78	58	129	35	2.38	0.047	1.3		2.89	0.094
701332	SW	29/04/2005	120	70	148	226	376	131	8.76	0.074	1.41		8.28	0.554
701333	SW	29/04/2005	120	118	216	158	247	130	5.14	1.96	5.19		13.2	1.4
701389	Eff	04/05/2640	168	184	233	251	345	174		1.63	7.33			
701392	Eff	28/04/2005	144	41	115	102	206	80	3.94	0.746	<2.92		5.65	0.261
701394	Eff	28/04/2005	144	174	494	168	333	170	9.20	9.5	3.85			
701395	Eff	28/04/2005	144	160	519	150	331	154	7.35	13.1	5.19			
701398	Eff	29/04/2005	120	108	265	126	234	107	7.30	1.77	5.57			
701399	Eff	29/04/2005	120	213	396	242	282	232	11.70	2.63	21.2			
701401	Eff	29/04/2005	120	102	246	221	322	147	5.50	<0.5	<2.92			
701402	Eff	29/04/2005	120	225	281	288	351	206	31.34	1.94	8.14			
701403	Eff	29/04/2005	120	171	343	268	426	220		2.01	<2.92			
701404	Eff	29/04/2005	120	211	551	153	320	193		10.1	5.69			
701410	Eff	29/04/2005	120	319	295	392	382	247	25.18	7.14	14.5			
701411	Eff	29/04/2005	120	111	169	118	180	109	3.81	<0.5	<2.92			
701429	Eff	29/04/2005	120	17	55	40	71	31	0.47	<0.03	<1			
701430	Eff	29/04/2005	120	320	367	357	414	270	19.95	5.31	13.2			
701431	Eff	29/04/2005	120	61	118	112	161	85	3.96	<0.5	<2.92			
701432	Eff	29/04/2005	120	127	191	117	172	107	7.44	2.14	6.94			
701433	Eff	29/04/2005	120	137	193	118	171	105	10.30	1.26	6.97			
701434	Eff	29/04/2005	120	119	192	127	184	112	5.98	5.65	5.66			
701435	Eff	29/04/2005	120	58	122	71	127	67	3.49	<0.5	<2.92			
701436	Eff	29/04/2005	120	231	559	332	550	205	48.64	1.6	18.2			
701449	Eff	29/04/2005	120	175	234	182	278	156	11.86	1	10.9			
701450	Eff	29/04/2005	120	217	275	266	347	204	12.35	1.73	14.4			
701451	Eff	29/04/2005	120	92	165	90	147	78		<0.5	3.82			

701452	Eff	29/04/2005	120	71	156	79	156	76	2.95	<0.5	<2.92			
707100	SW	16/05/2005	48	13	48	23	56		3.26	0.034	1.07			
707101	SW	16/05/2005	48	14	36	20	56	20	3.12	<0.03	<1			
707102	SW	16/05/2005	48	18	53	26	51	26	3.26	0.133	1.19		4.82	0.029
707103	SW	16/05/2005	48	19	48	30	69	29	2.83	0.055	1.21		4.9	0.077
707104	SW	16/05/2005	48	20	48	42	83	28	2.11	<0.03	<1		4.88	0.071
707105	SW	16/05/2005	48	21	51	40	82	29	3.63	<0.03	<1		4.8	0.47
707108	SW	16/05/2005	48	47	97	63	119	56	4.47	0.041	2.04		7.52	1.165
707109	SW	16/05/2005	48	61	102	97	161			0.033	1.26		13.3	0.739
707110	SW	16/05/2005	48	41	90	52	112	43	4.02	0.106	1.28		4.76	0.409
707111	SW	16/05/2005	48	17	62	38	84	27	3.63	0.059	<1		3.7	0.117
707112	SW	16/05/2005	48	15	52	26	83	25	1.84	<0.03	<1		3.75	0.034
707113	SW	16/05/2005	48	23	73	41	94	37		0.122	3.19		3.59	0.024
707116	SW	16/05/2005	48	25	80	58	115	47	4.63	<0.03	<1		4.52	0.22
707117	SW	16/05/2005	48	29	82	63	127	45	5.34	0.082	3.85		4.8	0.282
707118	SW	16/05/2005	48	39	93	50	114	45	5.77	<0.03	1.59		3.96	0.179
707119	SW	16/05/2005	48	31	88	61	140	48	3.69	0.05	1.37		4.67	0.025
707120	SW	16/05/2005	48	20	78	22	88	29	3.14	<0.03	1.38		2.56	<0.02
707121	SW	16/05/2005	48	38	102	59	135	48	4.89	<0.03	1.86		2.06	0.066
707122	SW	16/05/2005	48	40	92	67	130	51	4.07	<0.03	1.56		6.44	0.191
707123	SW	16/05/2005	48	66	135	97	167	83	5.66	0.563	2.29		9.34	0.759
707124	SW	16/05/2005	48	48	103	67	143	53	4.38	<0.03	2.52		4.63	0.113
707125	SW	16/05/2005	48	90	205	94	225	87	6.79	<0.03	7.93		0.744	0.037
707127	SW	16/05/2005	48	26	76	86	187	56		<0.03	<1		3.06	0.143
707128	SW	16/05/2005	48	47	102	69	137	57	5.02	<0.03	3.55		6.09	0.144
707129	SW	16/05/2005	48	37	80	50	102	38	2.02	0.076	1.11		4.21	0.138
707130	SW	16/05/2005	48	29	85	39	76	32	1.45	0.064	<1		1.99	<0.02
707131	SW	16/05/2005	48	16	33	43	85	28	1.49	<0.03	<1		5.81	0.022
707132	SW	16/05/2005	48	40	94	50	99	42	2.38	0.064	1.67		4.63	0.236
707133	SW	16/05/2005	48	43	112	51	108	44	2.61	0.076	1.63		4.47	0.283
707258	Eff	16/05/2005	48	257	428	324	417	235	14.03	3.42	12.7			
707259	Eff	16/05/2005	48	286	410	361	428	250	20.03	2.78	14.9			
707261	Eff	16/05/2005	48	185	284	221	314		8.88	<0.5	<2.92			
707266	Eff	16/05/2005	48	215	285	307	361	197	11.91	1.63	6.92			
707273	Eff	16/05/2005	48	256	414	363	434	249	12.63	3.1	3.3			
707280	Eff	16/05/2005	48	183	260	261	320		10.14	1.72	7.33			
707283	Eff	16/05/2005	48	205	349	311	401	224	11.20	0.5	4.7			
707286	Eff	16/05/2005	48	268	405	266	344	229	14.26	4.34	14.5			
707295	Eff	16/05/2005	48	287	399	335	404	239	16.47	4.27	15		27.1	1.26
707312	Eff	16/05/2005	48	256	336	302	350	218	14.08	5.43	16.1			
707314	Eff	16/05/2005	48	282	120	583	561	217	38.32		14.7			
707315	Eff	16/05/2005	48	177	339	288	397	194		<0.5	<2.92		11.1	5.57
707316	Eff	16/05/2005	48	129	197	149	234	125		<0.5	<2.92			

707322	Eff	16/05/2005	48	291	429	248	320	230		11.4	15.4			
707323	Eff	16/05/2005	48	544	1524	401	870	442	23.95	25.8	28.4			
707324	Eff	16/05/2005	48	158	373	211	399	182	10.82	1.8	<2.92			
707325	Eff	16/05/2005	48	987	2534	461	1830	570	21.79	39.5	26.7		<0.9	3.01
707330	Eff	16/05/2005	48	344	411	364	407	266	17.99	1.36	26.4			
715854	SW	05/06/2005	72	81	288	182	590	139	8.23	<0.03	3.76		0.216	
715855	SW	05/06/2005	72	72	187	138	273	100	6.70	0.109	2.92		6.7	
715856	SW	05/06/2005	72	80	175	137	279	92	5.96	0.185	2.24		6.29	
715857	SW	05/06/2005	72	52	147	110	226	76	5.98	0.125	1.55		7.31	
715858	SW	05/06/2005	72	45	154	118	234	73	18.47	0.067	1.91		6.93	
715859	SW	05/06/2005	72	55	149	126	240	85	7.12	0.082	1.74		6.62	
715878	SW	06/06/2005	48	23	97	83	191	51	2.76	0.034	<1		1.98	
715883	SW	06/06/2005	48	35	108	91	201	55	4.08	<0.03	<1		2.59	
715887	SW	06/06/2005	48	82	167	142	283	104	9.37	0.383	3.38		4.02	
715895	SW	06/06/2005	48	35	118	91	193	47	3.94	<0.03	2.46		1.84	
715897	SW	06/06/2005	48	112	262	290	384	132	20.27	0.89	3.74		4.81	
715905	SW	06/06/2005	48	45	109	95	211	66	5.98	0.052	1.76		4.88	
715918	SW	06/06/2005	48	544	1547	358	999	386	20.36	4.12	35.7		<0.196	
715919	SW	06/06/2005	48	43	110	94	176	62	5.11	0.034	2.13		6.85	6.88
715927	SW	06/06/2005	48	26	97	92	204	44	4.10	0.037	1.59		1.45	
715943	SW	06/06/2005	48	99	277	160	303	121	7.17	0.381	3.91		5.66	
715982	SW	06/06/2005	48	25	91	93	201	46	4.24	<0.03	1.85		1.6	
716023	SW	06/06/2005	48	51	144	86	171	63	4.95	0.119	3.17		6.7	
716024	SW	06/06/2005	48	46	130	104	195	70	4.87	0.048	1.76		6.27	
716025	SW	06/06/2005	48	39	100	82	167	56	4.20	<0.03	1.76		6.09	
716049	SW	06/06/2005	48	16	39	155	258	42	9.64	<0.03	<1		<0.196	<0.23
716050	SW	06/06/2005	48	19	55	124	234	38	7.94	<0.03	1.02		0.836	
716051	SW	06/06/2005	48	22	122	137	262	42	8.86	<0.03	<1		<0.196	
716052	SW	06/06/2005	48	15	75	144	266	42	8.96	<0.03	<1		<0.196	<0.23
716053	SW	06/06/2005	48	17	58	47	109	31	1.85	0.067	<1			
716054	SW	06/06/2005	48	28	90	53	126	35	2.44	0.391	1.44		1.49	
716055	SW	06/06/2005	48	24	80	61	139	36	2.72	<0.03	<1		1.85	
716064	SW	06/06/2005	48	46	118	101	202	67	5.98	0.042	1.43		7.18	
716065	SW	06/06/2005	48	33	92	71	141	48	4.66	0.121	1.36		8.38	
716066	SW	06/06/2005	48	26	62	62	122	38		<0.03	<1		7.76	
716083	SW	06/06/2005	48	49	127	97	198	66	5.57	0.075	2.41		6.51	
716234	Eff	05/06/2005	72	159	326	217	303	159	3.44	<0.5	<2.92		15.5	
716235	Eff	05/06/2005	72	230	418	270	382	208	9.47	5.51	9.95		19.9	
716236	Eff	05/06/2005	72	195	291	265	323	176	13.98	4.3	9.75		28.5	
716237	Eff	05/06/2005	72	193	389	247	395	184		<0.5	<2.92		11.2	
716238	Eff	05/06/2005	72	272	407	304	414	225	13.13	1.77	14		23	
716240	Eff	05/06/2005	72	251	385	290	406	213	14.36	1.97	11.5		26.3	

716241	Eff	05/06/2005	72	253	441	323	418		14.75	3.55	13			
716242	Eff	05/06/2005	72	162	444	252	433	185	14.26	<0.5	<2.92		4.6	4.7
716257	Eff	06/06/2005	48	281	731	156	464	208	6.57	3.74	40			
716258	Eff	06/06/2005	48	185	316	239	362	172	10.97	2.32	4.16			
716276	Eff	06/06/2005	48	226	387	290	392	201	11.21	1.54	11.7			
716277	Eff	06/06/2005	48	160	524	1428	856	216	7.98	1.51	<2.92			
716281	Eff	06/06/2005	48	298	355	403	463	232	17.95	3.14	11.5			
716282	Eff	06/06/2005	48	288	356	352	425	229	13.27	3.42	19.6			
716330	Eff	06/06/2005	48	195	496	281	417	178	8.92	1.89	4.38			
719144	SW	13/06/2005	48	29	114	141	297	64	4.01	<0.03	<1		1.06	
719145	SW	13/06/2005	48	44	92	176	329	88	5.15	<0.03	1.08		4.63	
719153	SW	13/06/2005	48	60	136	181	326	108	6.94	0.074	2		5.45	
719159	SW	13/06/2005	48	44	107	147	297	86	4.70	0.044	1.22		4.72	
719160	SW	13/06/2005	48	22	124	26	78	31	-1.65	0.103	1.07		5.75	
719164	SW	13/06/2005	48	13	44	21	64	20	-2.14	<0.03	<1		5.28	
719168	SW	13/06/2005	48	72	145	173	345	94	5.85	0.408	3.31		4.38	
719172	SW	13/06/2005	48	41	79	165	309	81	5.55	<0.03	<1		3.64	
719184	SW	13/06/2005	48	34	109	145	300	72	4.85	<0.03	1.11		2.35	
719187	SW	13/06/2005	48	30	133	37	89	38	-2.85	<0.03	1.05		5.92	
719197	SW	13/06/2005	48	22	112	36	74	30	-1.59	<0.03	<1		6.09	
719209	SW	13/06/2005	48	38	186	40	98	38	-0.82	<0.03	1.56		4.89	
719216	SW	13/06/2005	48	48	188	94	207	65	0.98	0.092	1.44		3.71	
719222	SW	13/06/2005	48	32	107	134	274	78	3.33	<0.03	1.29		3.92	
719224	SW	13/06/2005	48	44	116	97	210	65	0.79	0.039	1.78		3.31	
719236	SW	13/06/2005	48	59	156	109	252	84	2.48	<0.03	3.47		2.62	
719266	SW	13/06/2005	48	25	87	63	152	39	3.21	0.044	<1		2.58	
719267	SW	13/06/2005	48	19	62	65	173	35	2.28	<0.03	<1		1.28	
719269	SW	13/06/2005	48	13	34	28	71	27	0.83	<0.03	<1		5.15	
719270	SW	13/06/2005	48	15	43	26	70	27	1.06	<0.03	<1		5.14	
719271	SW	13/06/2005	48	13	38	37	100	30	1.13	<0.03	<1		3.42	
719272	SW	13/06/2005	48	15	26	21	63	27	0.19	<0.03	<1		5.49	
719274	SW	13/06/2005	48	20	51	64	126	38	1.79	<0.03	<1		4.18	
719275	SW	13/06/2005	48	17	53	57	135	31	1.94	<0.03	<1		2.48	
719442	Eff	13/06/2005	48	225	475	245	416	216	0.00	<0.5	<2.92			
719443	Eff	13/06/2005	48	226	329	288	367	207	10.65	<0.5	3.66			
719444	Eff	13/06/2005	48	227	436	346	438	217	11.68	2.92	2.96			
719446	Eff	13/06/2005	48	204	392	262	375	183	11.13	2.9	3.66			
719464	Eff	13/06/2005	48	96	209	171	269	120	9.67	<0.5	<2.92			
719469	Eff	13/06/2005	48	227	309	372	426	189	13.96	2.52	7.6			
719475	Eff	13/06/2005	48	228	333	388	426	212	12.84	1.11	6.67			
719479	Eff	13/06/2005	48	269	398	309	375	191	12.42	4.41	14.9			
719490	Eff	13/06/2005	48	591	2012	415	1376	460		35.9	25		<0.9	
719494	Eff	13/06/2005	48	287	328	382	509	253	13.85	<0.5	7.75			

719516	Eff	13/06/2005	48	275	349	373	461	252	11.03	0.63	10.9			
719517	Eff	13/06/2005	48	252	338	381	479	220	10.00	<0.5	<2.92			
719524	Eff	13/06/2005	48	238	302	345	419	211	14.10	4.29	5.34			
719527	Eff	13/06/2005	48	549	661	402	411	358	25.24	14.4	90			
719528	Eff	13/06/2005	48	249	434	369	461	237	12.02	1.56	4.06			
719529	Eff	13/06/2005	48	121	298	274	370	172	-0.70	6.04	<2.92			
719530	Eff	13/06/2005	48	280	431	280	341	217	10.30	3.43	10.6			
719531	Eff	13/06/2005	48	151	214	271	337	166	8.98	1.46	<2.92			
719532	Eff	13/06/2005	48	244	373	346	425	219	12.29	2.35	4.55			
719533	Eff	13/06/2005	48	219	322	289	346	190	9.25	1.59	4.82			
719534	Eff	13/06/2005	48	539	484	532	581	377	28.61	5.12	29.1			
719538	Eff	13/06/2005	48	251	373	371	447	245	8.77	<0.5	<2.92			
719539	Eff	13/06/2005	48	360	535	459	513	315	17.92	8.5	8.06			
719541	Eff	13/06/2005	48	348	466	446	476	183	19.55	5.8	22.4			
719543	Eff	13/06/2005	48	301	457	383	488	235	19.26	5.59	12			
725656	Eff	27/06/2005	48	30	73	56	118	40	4.32	<0.03	2.31		5.54	
725657	SW	27/06/2005	48	15	49	30	72	24	9.53	0.06	<1		6.66	
725658	SW	27/06/2005	48	21	62	30	83	29	5.28	0.232	1.34		6.05	
725659	SW	27/06/2005	48	17	43	32	64	27	1.52	0.265	1.08			
725660	SW	27/06/2005	48	20	51	24	80	27	1.93	0.377	<1			
725661	SW	27/06/2005	48	48	120	198	408	86	7.89	0.034	1.03		0.942	
725662	SW	27/06/2005	48	32	120	159	332	70	6.17	<0.03	<1		1.23	
725663	SW	27/06/2005	48	56	119	167	355	71	7.07	0.134	1.05		1.3	
725664	SW	27/06/2005	48	33	104	158	299	77	7.49	<0.03	<1		1.42	
725665	SW	27/06/2005	48	73	166	317	474	13	15.07	0.057	2.55		2.78	
725667	SW	27/06/2005	48	41	89	108	209	61	6.13	<0.03	<1		3.82	
725668	SW	27/06/2005	48	37	86	132	259	64	6.59	<0.03	<1		3.71	
725669	SW	27/06/2005	48	34	94	165	322	76	7.22	<0.03	1.05		1.46	
725670	SW	27/06/2005	48	36	119	142	279	69	5.78	<0.03	<1		3.07	
725671	SW	27/06/2005	48	37	116	146	282	72	6.85	<0.03	<1		3.03	
725672	SW	27/06/2005	48	40	151	217	398	94	8.97	<0.03	<1		1.1	
725673	SW	27/06/2005	48	36	103	143	286	74	7.57	<0.03	1.95		3.1	
725675	SW	27/06/2005	48	80	275	69	230	68	14.98	<0.03	3.3			
725676	SW	27/06/2005	48	61	151	78	218	89	2.97	0.08	3.53		3.36	
725677	SW	27/06/2005	48	36	101	61	175	60	3.80	0.122	<1		2.45	
725679	SW	27/06/2005	48	37	84	79	183	62	3.14	<0.03	<1		2.41	
725681	SW	27/06/2005	48	28	98	81	181	60	3.82	<0.03	1.13		2.39	
725682	SW	27/06/2005	48	69	177	86	177	82	3.45	0.062	1.58		4.11	
725683	SW	27/06/2005	48	62	177	85	199	98	3.77	0.062	1.72		4.12	
725684	SW	27/06/2005	48	30	77	67	152	44	3.84	<0.03	<1		3.95	
725685	SW	27/06/2005	48	28	102	53	136	59	2.10	0.047	<1		3.19	
725820	Eff	27/06/2005	48	215	311	291	353	183	14.14	4.83	9.52			
725823	Eff	27/06/2005	48	810	2370	774	1094	531		5.18	34.7			

725827	Eff	27/06/2005	48	321	389	367	445	246	17.60	2.4	15.9			
725834	Eff	27/06/2005	48	195	288	352	439	199	11.15	<0.5	<2.92			
725844	Eff	27/06/2005	48	171	333	377	472	193	11.69	<0.5	<2.92			
725852	Eff	27/06/2005	48	117	184	250	315	141	7.97	0.55	<2.92			
725854	Eff	27/06/2005	48	200	286	288	349	181	11.21	1	7.03			
725858	Eff	27/06/2005	48	161	304	207	271	143	8.20	1.13	6.26			
725863	Eff	27/06/2005	48	268	559	565	623	277	15.64	1.92	5.55			
725871	Eff	27/06/2005	48	172	324	231	310	162	18.49	3.33	4.41			
725875	Eff	27/06/2005	48	203	285	341	435	200	10.36	0.205	3.15		29.2	
725878	Eff	27/06/2005	48	248	309	398	452	221	17.75	0.7	10			
725886	Eff	27/06/2005	48	187	268	248	318	164	12.51	1.42	10.7			
725888	Eff	27/06/2005	48	220	457	255	457	215	7.08	<0.5	<2.92			
725889	Eff	27/06/2005	48	96	197	127	225	98	6.72	0.57	3.83			
725890	Eff	27/06/2005	48	306	917	326	740	283	18.58	29.9	16.3		<0.9	
725891	Eff	27/06/2005	48	138	257	190	299	140	9.17	1.11	<2.92			
725892	Eff	27/06/2005	48	233	588	354	609	253	12.47	9.38	3.82			
725899	Eff	27/06/2005	48	436	680	408	463	333	18.19	2.21	25.9		13	
725900	Eff	27/06/2005	48	243	327	284	363	215	13.89	6.1	9.48		18.6	
725902	Eff	27/06/2005	48	198	296	295	386	188	11.36	9.17	5.22		19.9	
725903	Eff	27/06/2005	48	228	656	358	661	246		1.08	<2.92			
725904	Eff	27/06/2005	48	158	234	207	292	148	12.48	2.7	5.29			
738590	SW	25/07/2005	864	21	32	96	208	37	3.93	<0.03	<1		<0.196	<0.02
738609	SW	25/07/2005	864	17	99	82	195	37	2.90	<0.03	<1			
738650	SW	25/07/2005	864	20	68	116	218	44	4.76	<0.03	<1		0.971	0.117
738651	SW	25/07/2005	864	24	72	104	222	46	4.98	<0.03	<1		1.53	0.033
738652	SW	25/07/2005	864	25	93	91	193	56	2.49	0.031	<1		1.72	0.067
738653	SW	25/07/2005	864	35	115	91	209	70	4.18	<0.03	1.19		3.12	0.079
738654	SW	25/07/2005	864	21	69	108	248	40	5.38	0.062	<1		3.17	0.056
738655	SW	25/07/2005	864	20	74	102	235	39	5.25	<0.03	<1		0.796	<0.02
738656	SW	25/07/2005	864	18	73	100	209	38	4.33	<0.03	<1		0.786	<0.02
738657	SW	25/07/2005	864	28	73	95	194	54	3.71	<0.03	<1		0.896	0.023
738658	SW	25/07/2005	864	17	82	103	214	46	4.37		<1			
738660	SW	25/07/2005	864	39	168	73	217	53	5.33		5.16			
738661	SW	25/07/2005	864	43	106	81	186	61	4.37		<1			
738665	SW	25/07/2005	864	185	213	350	384	169	12.10		5.3			
738667	SW	25/07/2005	864	18	76	98	220	42	4.16	<0.03	<1		1.35	0.028
738670	SW	25/07/2005	864	21	65	113	310	41	5.08	<0.03	<1		0.606	0.07
738671	SW	25/07/2005	864	16	104	134	280	48	6.00	<0.03	<1			
738672	SW	25/07/2005	864	19	108	127	271	44	5.71	<0.03	<1		<0.196	<0.02
738673	SW	25/07/2005	864	20	106	124	242	38	4.26	<0.03	<1		<0.196	<0.02
738674	SW	25/07/2005	864	11	39	43	109	22	1.36	<0.03	<1		1.09	<0.02
738675	SW	25/07/2005	864	21	80	104	224	41	4.43	<0.03	<1		0.536	<0.02

738689	SW	26/07/2005	840	37	158	211	371	88	9.14	0.103	3.59		2.35	0.133
738690	SW	26/07/2005	840	51	188	236	414	113	9.22	0.124	3.81		3.37	0.133
738691	SW	26/07/2005	840	72	194	369	520	133	17.58		6.69			
738698	SW	26/07/2005	840	70	110	367	536	136	15.82	0.293	6.81		5.68	0.22
738699	SW	26/07/2005	840	56	113	261	439	93	10.95	0.031	1.54		2.26	0.076
738700	SW	26/07/2005	840	65	109	366	542	137	16.71	0.267	6.25		5.42	0.192
738704	SW	26/07/2005	840	73	118	332	491	127	15.26	0.357	6.35		5.16	0.229
738705	SW	26/07/2005	840	59	141	205	397	110	8.77	0.078	3.04		2.51	0.143
738706	SW	26/07/2005	840	63	120	199	368	102	8.16	0.284	5.8		2.57	0.18
738707	SW	26/07/2005	840	51	136	185	358	93	8.00	0.075	2.09		2.75	0.109
738708	SW	26/07/2005	840	54	140	183	350	95	5.80	0.037	1.86		2.72	0.1
738867	Eff	24/07/2005	912	201	530	298	675	238	10.84	24.4	45.2			2.77
738881	Eff	25/07/2005	888	83	220	240	209	147	9.58	2.73	3.15			
738904	Eff	25/07/2005	888	107	237	142	274	113	6.91	0.63	3.73			
738905	Eff	25/07/2005	888	106	180	194	322	125	6.68	<0.03	<2.92			
738916	Eff	25/07/2005	888	109	235	243	361	136	8.25	<0.5	<2.92			
738917	Eff	25/07/2005	888	125	208	274	357	134	7.86	1.91	6.85			
738918	Eff	25/07/2005	888	195	605	271	676	245	12.08		15.7			
738919	Eff	25/07/2005	888	116	214	279	430	172	8.83		<3			
738920	Eff	25/07/2005	888	112	177	194	277	122	6.22		<3			
738924	Eff	25/07/2005	888	237	331	374	511	218	12.78	9.79	9.06			
738925	Eff	25/07/2005	888	37	102	96	185	58	4.70	0.051	<2.92		5.79	0.131
738926	Eff	25/07/2005	888	141	305	242	385	149	8.41	11.6	13			
738927	Eff	26/07/2005	840	73	221	183	306	117	5.54	1.12	13.9			
738928	Eff	26/07/2005	840	212	545	288	595	228	13.22	21.2	<2.92		<0.9	4.67
738948	SW	25/07/2005	888	19	68	107	248	40	4.63	<0.03	<1			
738949	SW	25/07/2005	888	22	116	113	232	44	5.10	0.047	1.01			
738956	SW	25/07/2005	888	44	149	73	203	57	5.36		<1			
738957	SW	25/07/2005	888	55	102	91	231	69	4.66		1.98			
760899	SW	12/09/2005	48	26	76	43	99	34	3.89	<0.03	<1		2.55	0.024
760900	SW	12/09/2005	48	25	43	39	66	27	5.42	<0.03	<1		9.27	0.077
760902	SW	12/09/2005	48	20	41	47	85	29	7.27	<0.03	<1		7.35	0.024
760903	SW	12/09/2005	48	38	79	76	141	48	5.47		<1			
760904	SW	12/09/2005	48	34	82	75	144	45	4.81	0.036	1.11		4.82	0.147
760905	SW	12/09/2005	48	25	78	73	141	46	5.24	<0.03	<1		5.08	0.147
760906	SW	12/09/2005	48	29	65	64	131	41	4.11	<0.03	<1		4.5	0.141
760907	SW	12/09/2005	48	27	71	64	124	41	6.11	<0.03	<1		3.41	0.146
760908	SW	12/09/2005	48	56	151	79	158	58	3.71	0.226	<1		4.55	0.811
760909	SW	12/09/2005	48	20	117	21	68	29	1.23		<1			
760910	SW	12/09/2005	48	19	62	29	75	25	1.14		<1			
760911	SW	12/09/2005	48	11	37	20	78	20	0.78		<1			
760913	SW	12/09/2005	48	27	84	55	109	42	1.88		<1			
760914	SW	12/09/2005	48	48	113	63	124	53	3.05	0.307	<1		7.76	0.899

760930	SW	12/09/2005	48	51	122	86	181	63	7.23	0.046	<1		2.68	0.509
760931	SW	12/09/2005	48	52	131	85	182	63	8.51	0.053	1.07		2.83	0.557
761066	Eff	12/09/2005	48	142	264	286	393	174	8.04	<0.5	<2.92			
761070	Eff	12/09/2005	48	220	315	337	405	197	13.60	<0.5	4.03			
761073	Eff	12/09/2005	48	252	547	377	410	208	17.05	0.97	<3.89			
761077	Eff	12/09/2005	48	2424	7327	868	4966	1590	133.40		507			
761086	Eff	12/09/2005	48	142	244	204	286	134	8.34	0.55	4.21			
761092	Eff	12/09/2005	48	197	289	272	346	170	13.06	2.27	11.3			
761107	Eff	12/09/2005	48	167	273	262	348	153	12.31		6.71			
761115	Eff	12/09/2005	48	149	252	270	394	161	9.08	<0.5	<2.92		14.1	<0.5
761118	Eff	12/09/2005	48	276	535	413	508	234	20.88	10.1	5.18			
761124	Eff	12/09/2005	48	175	341	327	457	181	8.61	1.08	<2.92			
761126	Eff	12/09/2005	48	243	293	339	448	222	14.85	6.81	10.6			
761128	Eff	12/09/2005	48	1239	3496	733	1863	785	28.28	69.9	41.1		<0.9	8.49
761130	Eff	12/09/2005	48	253	379	448	540	235	16.05	13.7	<4.87		17.4	6.25
761148	Eff	12/09/2005	48	391	786	520	579	273	30.53	3.17	26.4			
761153	Eff	12/09/2005	48	230	317	315	414	200	13.00	2.2	5.23			
761154	Eff	12/09/2005	48	194	255	347	392	182	15.22	<0.5	4.05			
761155	Eff	12/09/2005	48	178	272	280	364	167	11.76	<0.5	3.65			
761156	Eff	12/09/2005	48	234	320	382	534	202	16.10	6.6	<2.92			8.05
761157	Eff	12/09/2005	48	230	276	313	411	198	15.65	5.51	7.39			
761158	Eff	12/09/2005	48	235	306	337	457	199	13.82	3.35	8.85			
761159	Eff	12/09/2005	48	180	228	261	366	191	7.26	<0.5	<2.92			
761160	Eff	12/09/2005	48	225	658	331	526	220	13.08	21.8	<3.89			
761161	Eff	12/09/2005	48	246	418	290	397	195	15.92	6.91	11			
761162	Eff	12/09/2005	48	198	309	300	385	188	10.09	1.73	3.33		20.4	<0.5
761168	Eff	12/09/2005	48	321	523	382	450	257	24.92	9.37	20.1			
761169	Eff	12/09/2005	48	335	788	300	480	257	19.99		21.6			
761170	Eff	12/09/2005	48	185	543	286	576	205	13.90	12.8	<2.92			
761171	Eff	12/09/2005	48	611	1972	565	1524	530	26.27		22.5			
761173	Eff	12/09/2005	48	91	271	296	429	149	16.42		<2.92			
761175	Eff	12/09/2005	48	178	323	289	399	173	20.07		5.66			
761179	Eff	12/09/2005	48	226	578	390	548	200	10.72	1.4	1.65			
761180	Eff	12/09/2005	48	171	291	331	469	191	16.48	0.52	<2.92			
761205	Eff	12/09/2005	48	321	366	469	520	274	26.31	8.38	13.8			
761213	Eff	12/09/2005	48	82	227	655	651	150	12.98		<2.92			
771858	Eff	10/10/2005	48	191	333	306	366	161	10.66	<0.5	1.85			
771891	SW	10/10/2005	48	25	67	123	244	49	5.52	<0.03	<1		2.9	0.054
771898	SW	10/10/2005	48	557	1344	760	2189	495	32.38	31	32.2		<0.9	1.15
771902	SW	10/10/2005	48	22	84	123	256	49	5.89	<0.03	<1		1.85	0.086
771968	SW	10/10/2005	48	22	80	86	182	41	3.34	<0.03	<1		1.68	0.423
771979	SW	10/10/2005	48	61	115	116	171	59	4.91	<0.03	1.99		12.2	0.73

771980	SW	10/10/2005	48	25	54	86	191	45	3.83	<0.03	<1		2.23	<0.02
771981	SW	10/10/2005	48	30	94	94	192	45	3.75	<0.03	<1		3.06	<0.02
771982	SW	10/10/2005	48	76	195	201	415	96	9.09	<0.03	<1		1.08	0.177
771983	SW	10/10/2005	48	25	67	151	298	65	6.47	<0.03	<1		2.11	0.06
771984	SW	10/10/2005	48	66	149	197	448	103	7.98	0.035	1.17		0.704	0.214
771985	SW	10/10/2005	48	55	140	192	417	100	8.25	0.049	1.53		0.742	0.175
771986	SW	10/10/2005	48	86	177	211	351	104	14.60	0.207	2.45		2.86	0.243
771987	SW	10/10/2005	48	26	103	84	189	48	6.65	0.037	<1		2.6	0.076
771989	SW	10/10/2005	48	25	76	70	154	48		<0.03	<1		2.51	0.061
771990	SW	10/10/2005	48	27	75	84	193	41	4.74	<0.03	<1		2.71	0.058
771991	SW	10/10/2005	48	40	101	87	191	48	5.63	<0.03	<1		2.71	0.082
772000	Eff	10/10/2005	48	313	626	449	556	262	17.96	15.5	5.9			
772002	SW	10/10/2005	48	40	96	112	235	61	7.25	<0.03	<1		3.06	0.193
772003	SW	10/10/2005	48	65	187	142	292	100	9.27	0.03	<1		5.16	1.28
772005	SW	10/10/2005	48	52	152	138	267	82	6.78	0.058	1.1		4.12	0.788
772006	SW	10/10/2005	48	51	132	106	228	72		0.031	<1		2.78	2.79
772007	SW	10/10/2005	48	117	286	492	447	128	9.43	0.985	1.95		3.72	2.4
772008	SW	10/10/2005	48	27	93	84	177	45	3.52	0.048	1.19		3.59	<0.02
772068	SW	10/10/2005	48	13	73	76	178	31	3.19	<0.03	<1		0.606	<0.02
772069	SW	10/10/2005	48	22	63	77	159	38	3.34	<0.03	<1		1.51	0.234
772071	SW	10/10/2005	48	22	70	76	171	31	3.33	<0.03	<1		1.22	0.167
772073	SW	10/10/2005	48	35	82	156	316	75	9.37	0.079	1.07		7.05	0.045
772074	SW	10/10/2005	48	195	472	362	583	191	33.70	26	<1		<0.196	3.85
772075	SW	10/10/2005	48	25	80	76	199	43	4.06	<0.03	1.07		1.63	<0.02
772104	Eff	10/10/2005	48	432	522	576	624	299	30.42	18.2	17			
772105	Eff	10/10/2005	48	160	217	290	314	136	10.13	<0.5	<2.92			
772115	Eff	10/10/2005	48	280	417	452	549	227	18.56	1.98	8.38			
772123	Eff	10/10/2005	48	400	656	440	527	289	24.88	<0.5	5.83			
772130	Eff	10/10/2005	48	223	517	373	541	219	10.61	0.9	3.32			
772137	Eff	10/10/2005	48	330	502	434	485	260	20.75	11.7	9.97			
772155	Eff	10/10/2005	48	344	673	484	501	277	20.73	2.64	14.4			
772156	Eff	10/10/2005	48	183	276	324	392	161	11.85	<0.5	<2.92			
772158	Eff	10/10/2005	48	176	275	292	389	164	10.55	<0.5	<2.92			
772159	Eff	10/10/2005	48	192	262	253	338	163	14.74		5.64			
772161	Eff	10/10/2005	48	544	1865	458	971	460	21.36	23.2	16.8		<0.9	<0.5
772163	Eff	10/10/2005	48	244	362	373	506	210	16.32		7.23			
772164	Eff	10/10/2005	48	140	188	230	282	126	7.16	<0.5	<2.92			
772204	Eff	10/10/2005	48	138	227	282	346	141	9.34	<0.5	<2.92			
772205	Eff	10/10/2005	48	247	692	1631	1119	240	11.97	2.76	<3.89			
772206	Eff	10/10/2005	48	275	324	431	452	224	22.00	3.57	10.6			
788333	Eff	18/11/2005	120	1119	5790	480	3481	1180	10688.00	3.19	50	1200		1.07
788336	Eff	21/11/2005	48	307	283	504	599	262	11.30	19.6	7.86	57	5.41	11.4

788337	SW	21/11/2005	48	2060	7291	790	2940	1360	41.37	29.1	49.2	216		
788346	SW	18/11/2005	120	113	304	235	391	124	3.80	0.656	1.37	23	8.58	0.299
788348	SW	18/11/2005	120	46	124	202	408	96	2.91	0.042	2	39	4.96	0.076
788349	SW	18/11/2005	120	53	124	139	275	71	3.05	0.185	1.26	<12	7.35	0.212
788350	SW	18/11/2005	120	44	111	118	248	54	2.89	0.058	<1	<12	9.55	0.132
788353	SW	18/11/2005	120	73	147	244	393	109	5.36	<0.03	4.25	28	7.43	0.116
788354	SW	18/11/2005	120	26	46	94	196	49		0.071	1.04	<12	8.58	<0.02
788358	SW	18/11/2005	120	35	97	114	223	58	1.69	<0.03	1.13	<12	7.25	0.191
788361	SW	18/11/2005	120	54	118	166	314	80	1.63	0.797	1.24	15	7.59	0.226
788362	SW	18/11/2005	120	42	97	226	455	104	2.67	0.174	<1	19	7.21	0.151
788363	SW	18/11/2005	120	41	126	143	279	73	1.18	0.477	1.7	13	7.13	0.192
788364	Eff	20/11/2005	72	431	885	510	700	273	32.15	35.9	82	258		4.99
788365	Eff	20/11/2005	72	245	347	266	358	174	7.51	7.41	14.4	56		1.89
788366	Eff	20/11/2005	72	266	343	274	341	181	9.02	6.91	19.4	76		2
788378	SW	21/11/2005	48	55	89	152	300	76	2.41	0.058	1.37	13	3.98	0.204
788387	SW	21/11/2005	48	36	138	121	211	52	2.40	0.106	<1	13	1.29	0.097
788406	SW	21/11/2005	48	38	83	91	182	52	1.41	0.738	1.32	<12	7.01	0.4
788408	Eff	21/11/2005	48	258	666	607	1245	476	10.97	<0.5	2.14	48		
788410	SW	21/11/2005	48	28	93	142	258	63	2.73	0.06	1.21	14	7.51	0.036
788416	SW	21/11/2005	48	37	100	157	297	71	2.14	0.058	1.63	18	5.71	0.027
788438	SW	21/11/2005	48	36	80	165	337	74	3.00	0.097	1.15	16	3.99	0.035
788440	SW	21/11/2005	48	42	110	120	227	64	1.54	0.137	1.36	<12	9.36	0.183
788442	SW	21/11/2005	48	49	120	120	236	79	0.89	0.092	1.57	13	9.8	1.06
788446	SW	21/11/2005	48	52	108	334	639	122	5.59	0.67	1.22	26	1.33	0.025
788460	SW	21/11/2005	48	44	107	194	339	83	4.25	0.035	1.87	20	4.46	0.456
788468	SW	21/11/2005	48	81	217	171	272	103	4.38	0.172	1.74	18	11.6	0.558
788471	SW	21/11/2005	48	58	143	114	225	71	1.85	0.097	1.6	14	10.6	1.23
788474	SW	21/11/2005	48	42	96	246	499	99	5.89	0.183	1.47	24	2.41	0.036
788487	SW	21/11/2005	48	67	121	227	501	108	4.34	<0.03	1.73	23	1.15	<0.02
788489	SW	21/11/2005	48	83	175	178	325	110	3.62	1.95	1.95	22	7.7	0.566
788493	SW	21/11/2005	48	123	270	203	323	123	3.75	0.07	2.54	23	10.4	0.609
788495	SW	21/11/2005	48	54	158	241	510	109	5.32	0.04	1.51	23	1.11	0.029
788498	SW	21/11/2005	48	36	86	141	270	76	0.91	0.195	1.95	14	9.47	0.433
788508	Eff	21/11/2005	48	83	266	134	310	89	4.71	4.21	4.32	nr		0.835
788510	Eff	21/11/2005	48	130	331	496	864	210	15.02	8.82	45.6	771		1.42
788516	Eff	21/11/2005	48	69	173	387	845	165	7.05	2.16	<4.87	49		0.681
788518	SW	21/11/2005	48	52	112	155	290	86	2.72	0.065	1.32	14	7.91	0.244
788595	SW	22/11/2005	24	44	142	94	233	66	3.08	0.089	1.07	16	2.1	0.03
788597	SW	22/11/2005	24	43	158	104	236	68	3.37	0.093	<1	16	2.26	0.042
788598	SW	22/11/2005	24	41	154	99	216	69	3.51	0.079	<1	17	2.23	0.038
788612	SW	21/11/2005	48	76	184	217	401	125	4.24	0.263	1.43	24	6.79	0.34
788613	SW	21/11/2005	48	76	213	180	336	115	3.47	0.038	1.61	19	7.98	0.378
788721	Eff	21/11/2005	48	1464	4291	1243	1890	880	39.60	59.9	46.2	198		11.2

788739	Eff	21/11/2005	48	371	464	282	353	240	9.73	11.3	36.8	97		
788752	Eff	21/11/2005	48	398	524	361	446	278	12.04	18.7	27	94		
788763	Eff	21/11/2005	48	611	1602	425	694	350	14.16	9.37	39.6	148	0.71	8.6
788784	Eff	18/11/2005	120	26	40	78	152	47	1.75	<0.5	<2.92	12		<0.5
788788	Eff	20/11/2005	72	12	91	42	125	31		0.614	<2.92	5		0.03
788789	Eff	21/11/2005	48	40	153	131	244	72	0.51	0.187	<2.92	17		0.359
788795	Eff	21/11/2005	48	90	309	205	537	188	6.17	<0.5	<2.92	287		0.101
788800	Eff	21/11/2005	48	29	136	61	179	43	2.15	<0.03	<2.92	13		0.023
788801	Eff	21/11/2005	48	158	259	414	433	106	26.57	1.3	7.29	133		
788810	Eff	21/11/2005	48	146	461	65	183	92	6.71		20.1	62		
806019	Eff	15/01/2006	72	239	294	303	344	186	27.58	1.45	8.92			
806020	Eff	15/01/2006	72	180	300	263	426	165	16.70	4.77	14.5			
806021	Eff	15/01/2006	72	117	179	252	390	137	12.18	1.03	5.56			
806022	Eff	15/01/2006	72	215	333	269	362	184	20.67	3.44	8.45			
806023	Eff	15/01/2006	72	139	245	202	310	129	7.59	0.56	<2.92			
806024	Eff	15/01/2006	72	135	224	207	286	121	10.33	<0.5	<2.92			
806027	Eff	15/01/2006	72	78	143	191	247	99	7.97		<2.92			
806029	Eff	15/01/2006	72	87	173	111	167	75	6.08	<0.5	<2.92			
806031	Eff	16/01/2006	48	190	294	222	324	157	12.78	2.06	6.28			
806033	Eff	16/01/2006	48	107	191	155	258	85	8.08	<0.5	4.44			
806034	Eff	16/01/2006	48	239	350	328	423	182	16.80	4.13	7.75			
806043	Eff	16/01/2006	48	201	304	252	370	175	15.37	4.85	9.45			
806075	Eff	16/01/2006	48	226	401	264	383	183	16.38		10.4			
806079	Eff	16/01/2006	48	305	682	238	348	207	25.96	10.9	28.7			
806080	Eff	16/01/2006	48	155	309	141	239	112	10.10	5.34	12.2			
806081	Eff	16/01/2006	48	51	110	88	158	51	4.47	<0.5	<2.92			
806084	Eff	16/01/2006	48	116	200	217	316	135	8.99	1.51	<2.92		23.6	0.55
806087	Eff	16/01/2006	48	269	524	266	400	210	20.03	<0.5	9.95			
806088	Eff	16/01/2006	48	68	224	116	266	77	0.89	<0.5	<2.92			
806106	Eff	16/01/2006	48	211	312	306	369	173	14.79	8.66	7.38			
806108	SW	16/01/2006	48	27	56	110	206	56	2.32	<0.5	<2.92			
806109	Eff	16/01/2006	48	210	569	165	350	154	9.64	12.2	6.07			
806110	Eff	16/01/2006	48	219	560	345	542	179	23.05	12.8	10.6			
806111	Eff	16/01/2006	48	216	342	248	376	172	18.08	8.5	13.3			
815971	SW	13/02/2006	72	59	169	159	306	91	5.60	0.132	1.49		8.98	0.906
815973	SW	13/02/2006	48	20	35	90	196	47	2.06	<0.03	<1		2.71	0.039
815974	Eff	13/02/2006	48	107	175	160	216	92	3.25	<0.5	3			
815976	SW	13/02/2006	48	38	73	71	154	39	2.29	0.132	1.2		6.81	0.075
815977	Eff	13/02/2006	72	346	1118	162	820	274	12.27	0.166	>26.6		<0.2	|
815980	SW	13/02/2006	48	84	156	268	470	125	12.70	0.355	4.14		5.27	0.224
815981	SW	13/02/2006	72	23	48	53	122	29	2.04	0.047	<1		7.04	0.049
815982	SW	13/02/2006	48	9	15	26	60	19		<0.03	<1		<7.29	0.03

815983	SW	13/02/2006	72	29	65	80	170	41	2.41	0.168	1.23		6.82	0.093
815984	Eff	13/02/2006	48	269	741	302	587	239	11.83		4.88			
815986	Eff	12/02/2006	72	419	1347	343	916	346	14.49	25.7	23.5		<0.9	0.75
815988	Eff	12/02/2006	72	218	617	291	574	201	11.19	14.8	<2.92			
815989	SW	12/02/2006	96	16	56	43	110	30	0.77	0.046	1.94		3.65	0.03
815992	Eff	12/02/2006	72	399	1228	367	870	326	15.60		16.5			
815995	SW	12/02/2006	72	28	68	66	128	43	2.09	0.117	1.18		6.81	0.238
815996	Eff	12/02/2006	72	195	298	205	293	146	9.31	4.11	9.96			
815998	Eff	13/02/2006	48	259	388	290	390	198	17.74	<5	10.9			
815999	Eff	13/02/2006	48	221	474	322	431	192	12.20	12.6	3.94			
816000	Eff	13/02/2006	48	242	374	278	361	179	15.82	6.86	13.3			
816001	Eff	13/02/2006	48	163	274	292	428	145	9.28	<0.5	3.02			
816004	SW	13/02/2006	48	18	61	49	118	29	1.81	0.099	1.08		2.99	0.1
816005	SW	13/02/2006	48	6	21	16	59	16	0.95	<0.03	<1		<2.07	<0.02
816008	SW	13/02/2006	72	20	72	69	166	33	10.86	0.046	1.12		2.16	<0.02
816009	Eff	13/02/2006	72	30	106	147	271	56	5.53	0.209	1.2		1.86	<0.02
816018	Eff	13/02/2006	48	192	298	270	355	155	9.37	<0.5	<2.92			
816020	Eff	13/02/2006	48	325	585	338	516	238	20.27	16.6	16.3			
816021	Eff	13/02/2006	48	182	303	171	283	129	6.54		9.38			
816022	Eff	13/02/2006	48	202	400	277	419	186	9.52		<2.92			
816025	SW	13/02/2006	72	16	39	52	127	28	0.77	<0.03	1.41		4.5	0.056
816026	SW	13/02/2006	72	20	75	96	145	36	1.86	0.039	1.33		4.99	0.102
816027	SW	13/02/2006	48	26	93	88	166	56	0.76	0.044	1.12		5.62	0.072
816029	SW	13/02/2006	48	58	172	101	228	78	1.52	<0.03	1.09		7.53	0.149
816030	SW	13/02/2006	48	27	97	106	226	67	2.25	<0.03	<1		3.55	0.046
816031	SW	13/02/2006	72	79	330	234	475	137	4.75	7.63	<1	20		

APPENDIX 3

						Fluorescence Units (AFU)				mgl-1				CFU	
Name	Fraction	Day	Temp	Light	Dilution	T1Int	T2Int	Cint	Aint	DO	Co	TOC	pH	10-1 YEA	10-1 R2A
1	3.0	1	11	L	0	36	85	88	190	10.4	650		8.06		
1	3.0	1	11	L	0	36	115	81	187	10.4	650		8.06		
1	3.0	1	11	L	0	31	85	81	201	10.4	650		8.06		
1	1.0	1	11	L	0	40	112	93	206	10.4	650	2.97	8.06		
1	1.0	1	11	L	0	42	132	90	202	10.4	650	2.97	8.06		
1	1.0	1	11	L	0	39	118	94	217	10.4	650	2.97	8.06		
1	2.0	1	11	L	0	37	109	91	195	10.4	650	1.63	8.06		
1	2.0	1	11	L	0	42	106	93	199	10.4	650	1.63	8.06		
1	2.0	1	11	L	0	37	95	91	209	10.4	650	1.63	8.06		
1	3.0	2	11	L	0	39	100	85	205	7.47	611	2.94	8.24		
1	3.0	2	11	L	0	35	95	85	207	7.47	611	2.94	8.24		
1	3.0	2	11	L	0	32	95	85	212	7.47	611	2.94	8.24		
1	1.0	2	11	L	0	39	113	95	219	9.42	644	5.40	8.2		
1	1.0	2	11	L	0	41	100	93	210	9.42	644	5.40	8.2		
1	1.0	2	11	L	0	42	109	95	226	9.42	644	5.40	8.2		
1	1.0	2	11	L	0	41	103	98	212	9.28	641	4.75	8.14		
1	1.0	2	11	L	0	46	110	95	208	9.28	641	4.75	8.14		
1	1.0	2	11	L	0	41	127	96	209	9.28	641	4.75	8.14		
1	2.0	2	11	L	0	47	106	90	212	8.43	637	4.69	8.18		
1	2.0	2	11	L	0	46	110	90	225	8.43	637	4.69	8.18		
1	2.0	2	11	L	0	43	121	94	230	8.43	637	4.69	8.18		
1	3.0	3	11	L	0										
1	3.0	3	11	L	0										
1	3.0	3	11	L	0										
1	1.0	3	11	L	0	44	109	102	231	10.37	641	4.28	8.28	1	
1	1.0	3	11	L	0	48	110	101	231	10.37	641	4.28	8.28	1	
1	1.0	3	11	L	0	48	106	103	237	10.37	641	4.28	8.28	1	
1	1.0	3	11	L	0	41	119	97	219	10.32	642	4.77	8.25	1	
1	1.0	3	11	L	0	43	131	104	225	10.32	642	4.77	8.25	1	
1	1.0	3	11	L	0	52	116	103	224	10.32	642	4.77	8.25	1	
1	2.0	3	11	L	0	59	117	98	224	8.71	637	4.22	8.15		
1	2.0	3	11	L	0	57	129	98	236	8.71	637	4.22	8.15		
1	2.0	3	11	L	0	51	129	97	222	8.71	637	4.22	8.15		
1	3.0	4	11	L	0									7	
1	3.0	4	11	L	0									7	
1	3.0	4	11	L	0									7	
1	1.0	4	11	L	0	56	142	99	218	12.25	634	2.87	8.3	4	1
1	1.0	4	11	L	0	68	142	105	226	12.25	634	2.87	8.3	4	1
1	1.0	4	11	L	0	62	141	102	225	12.25	634	2.87	8.3	4	1
1	1.0	4	11	L	0	52	124	108	280	11.09	636	2.00	8.35	4	1
1	1.0	4	11	L	0	43	116	117	264	11.09	636	2.00	8.35	4	1
1	1.0	4	11	L	0	52	117	112	277	11.09	636	2.00	8.35	4	1
1	2.0	4	11	L	0	56	114	106	235	7.54	637		8.12		
1	2.0	4	11	L	0	39	106	94	225	7.54	637		8.12		
1	2.0	4	11	L	0	54	128	93	236	7.54	637		8.12		
1	3.0	5	11	L	0									18	
1	3.0	5	11	L	0									18	
1	3.0	5	11	L	0									18	
1	1.0	5	11	L	0	62	155	112	256	12.74	625		8.5	9	9
1	1.0	5	11	L	0	69	148	114	263	12.74	625		8.5	9	9
1	1.0	5	11	L	0	60	146	108	254	12.74	625		8.5	9	9
1	1.0	5	11	L	0	58	144	107	243	12.66	629		8.5	9	9
1	1.0	5	11	L	0	63	138	107	251	12.66	629		8.5	9	9
1	1.0	5	11	L	0	57	134	99	231	12.66	629		8.5	9	9
1	2.0	5	11	L	0	57	133	99	223	7.8	624		8.03	5	
1	2.0	5	11	L	0	51	134	93	238	7.8	624		8.03	5	
1	2.0	5	11	L	0	55	114	101	222	7.8	624		8.03	5	
1	3.0	12	11	L	0	63	146	96	231	11.16	607	6.42	8.39	38	
1	3.0	12	11	L	0	62	151	93	227	11.16	607	6.42	8.39	38	

1	3.0	12	11	L	0	67	145	94	234	11.16	607	6.42	8.39	38	
1	1.0	12	11	L	0	76	199	115	320	25.54	472	10.35	8.69	41	69
1	1.0	12	11	L	0	80	167	111	323	25.54	472	10.35	8.69	41	69
1	1.0	12	11	L	0	69	173	112	308	25.54	472	10.35	8.69	41	69
1	1.0	12	11	L	0	76	162	103	287	24.57	403	11.81	8.85	41	69
1	1.0	12	11	L	0	72	167	105	299	24.57	403	11.81	8.85	41	69
1	1.0	12	11	L	0	74	162	104	265	24.57	403	11.81	8.85	41	69
1	2.0	12	11	L	0	57	139	101	236	8.51	642	7.60	8.11	13	25
1	2.0	12	11	L	0	57	130	101	228	8.51	642	7.60	8.11	13	25
1	2.0	12	11	L	0	63	128	98	240	8.51	642	7.60	8.11	13	25
1	3.0	19	11	L	0	40	113	94	216	12.37	605	5.85	8.52	62	1
1	3.0	19	11	L	0	39	126	97	229	12.37	605	5.85	8.52	62	1
1	3.0	19	11	L	0	36	101	94	217	12.37	605	5.85	8.52	62	1
1	1.0	19	11	L	0	119	272	107	274	24.41	422	14.05	10.43	65	141
1	1.0	19	11	L	0	119	274	104	265	24.41	422	14.05	10.43	65	141
1	1.0	19	11	L	0	118	261	104	279	24.41	422	14.05	10.43	65	141
1	1.0	19	11	L	0	90	217	107	255	23.97	415	13.63	10.48	65	141
1	1.0	19	11	L	0	99	218	101	257	23.97	415	13.63	10.48	65	141
1	1.0	19	11	L	0	92	220	106	262	23.97	415	13.63	10.48	65	141
1	2.0	19	11	L	0	66	151	106	267	15.11	573	10.67	8.61	21	132
1	2.0	19	11	L	0	68	151	107	284	15.11	573	10.67	8.61	21	132
1	2.0	19	11	L	0	65	159	107	286	15.11	573	10.67	8.61	21	132
1	3.0	26	11	L	0	155	324	95	254	25.17	368	14.30	10.13	83	2
1	3.0	26	11	L	0	155	332	90	246	25.17	368	14.30	10.13	83	2
1	3.0	26	11	L	0	150	337	102	250	25.17	368	14.30	10.13	83	2
1	1.0	26	11	L	0	90	228	114	286	14.76	433	13.18	10.58	80	300
1	1.0	26	11	L	0	96	217	113	318	14.76	433	13.18	10.58	80	300
1	1.0	26	11	L	0	94	223	112	293	14.76	433	13.18	10.58	80	300
1	1.0	26	11	L	0	86	203	113	281	15.11	432	11.54	10.61	80	300
1	1.0	26	11	L	0	81	205	109	267	15.11	432	11.54	10.61	80	300
1	1.0	26	11	L	0	88	187	111	291	15.11	432	11.54	10.61	80	300
1	2.0	26	11	L	0	152	335	101	269	21.65	385	16.10	10.32	29	266
1	2.0	26	11	L	0	160	361	98	278	21.65	385	16.10	10.32	29	266
1	2.0	26	11	L	0	147	349	101	284	21.65	385	16.10	10.32	29	266
2	1.0	1	11	L	0	44	130	78	172	10.58	734	4.86	7.82		
2	1.0	1	11	L	0	47	118	81	181	10.58	734	4.86	7.82		
2	1.0	1	11	L	0	43	119	80	178	10.58	734	4.86	7.82		
2	2.0	1	11	L	0	42	124	81	174	10.58	738		7.9		
2	2.0	1	11	L	0	56	133	86	175	10.58	738		7.9		
2	2.0	1	11	L	0	55	128	80	184	10.58	738		7.9		
2	3.0	1	11	L	0	37	125	75	160	10.58	724		8.07		
2	3.0	1	11	L	0	48	125	75	182	10.58	724		8.07		
2	3.0	1	11	L	0	48	133	75	170	10.58	724		8.07		
2	1.0	2	11	L	0	32	116	83	186	8.33	732	4.65	8.03		
2	1.0	2	11	L	0	41	117	84	176	8.33	732	4.65	8.03		
2	1.0	2	11	L	0	48	128	79	180	8.33	732	4.65	8.03		
2	2.0	2	11	L	0	44	127	83	196	8.15	738	4.50	8.07		
2	2.0	2	11	L	0	49	119	86	181	8.15	738	4.50	8.07		
2	2.0	2	11	L	0	52	122	80	188	8.15	738	4.50	8.07		
2	3.0	2	11	L	0	49	148	79	175	6.8	738	4.08	8.18		
2	3.0	2	11	L	0	46	145	80	183	6.8	738	4.08	8.18		
2	3.0	2	11	L	0	50	120	80	181	6.8	738	4.08	8.18		
2	3.0	2	11	L	0	55	133	82	182	6.79	725	4.01	8.21		
2	3.0	2	11	L	0	63	131	81	200	6.79	725	4.01	8.21		
2	3.0	2	11	L	0	57	128	80	206	6.79	725	4.01	8.21		
2	1.0	3	11	L	0	53	111	80	176	8.47	737		8.05		
2	1.0	3	11	L	0	43	115	82	180	8.47	737		8.05		
2	1.0	3	11	L	0	47	115	82	178	8.47	737		8.05		
2	2.0	3	11	L	0	43	122	79	175	8.26	735		8.04		
2	2.0	3	11	L	0	48	140	83	171	8.26	735		8.04		
2	2.0	3	11	L	0	49	119	78	173	8.26	735		8.04		
2	3.0	3	11	L	0	53	121	80	183	7.07	737		8.18		
2	3.0	3	11	L	0	46	117	79	183	7.07	737		8.18		
2	3.0	3	11	L	0	50	122	86	195	7.07	737		8.18		
2	3.0	3	11	L	0	49	124	82	180	7.06	734		8.21		

2	3.0	3	11	L	0	47	131	82	179	7.06	734		8.21		
2	3.0	3	11	L	0	46	124	79	183	7.06	734		8.21		
2	1.0	4	11	L	0	50	126	78	191	9.6	739	1.88	8.17	1	3
2	1.0	4	11	L	0	49	117	81	188	9.6	739	1.88	8.17	1	3
2	1.0	4	11	L	0	49	121	80	180	9.6	739	1.88	8.17	1	3
2	2.0	4	11	L	0	50	126	82	178	8.63	744	1.19	8.16		20
2	2.0	4	11	L	0	54	141	80	180	8.63	744	1.19	8.16		20
2	2.0	4	11	L	0	46	126	79	188	8.63	744	1.19	8.16		20
2	3.0	4	11	L	0	44	132	83	183	7.19	739	4.26	8.21		
2	3.0	4	11	L	0	47	121	80	198	7.19	739	4.26	8.21		
2	3.0	4	11	L	0	52	135	80	185	7.19	739	4.26	8.21		
2	3.0	4	11	L	0	44	122	84	187	7.21	737	1.67	8.24		
2	3.0	4	11	L	0	45	122	80	185	7.21	737	1.67	8.24		
2	3.0	4	11	L	0	54	128	80	186	7.21	737	1.67	8.24		
2	1.0	5	11	L	0	51	124	83	188	10.67	739	5.74	8.26		14
2	1.0	5	11	L	0	56	118	84	189	10.67	739	5.74	8.26		14
2	1.0	5	11	L	0	53	117	85	202	10.67	739	5.74	8.26		14
2	2.0	5	11	L	0	61	120	91	200	7.06	746	5.01	8.1		2
2	2.0	5	11	L	0	49	130	86	193	7.06	746	5.01	8.1		2
2	2.0	5	11	L	0	51	125	91	204	7.06	746	5.01	8.1		2
2	3.0	5	11	L	0	46	125	77	183	7.34	741	4.36	8.11		1
2	3.0	5	11	L	0	46	125	81	194	7.34	741	4.36	8.11		1
2	3.0	5	11	L	0	43	123	78	188	7.34	741	4.36	8.11		1
2	3.0	5	11	L	0	49	115	79	188	7.1	736	4.69	8.19		1
2	3.0	5	11	L	0	46	135	81	192	7.1	736	4.69	8.19		1
2	3.0	5	11	L	0	53	115	82	182	7.1	736	4.69	8.19		1
2	1.0	12	11	L	0	81	156	88	207	21.45	590	7.30	8.38	45	62
2	1.0	12	11	L	0	76	147	90	203	21.45	590	7.30	8.38	45	62
2	1.0	12	11	L	0	78	158	85	209	21.45	590	7.30	8.38	45	62
2	2.0	12	11	L	0	49	87	88	220	6.47	745	5.62	7.89	24	72
2	2.0	12	11	L	0	46	97	85	212	6.47	745	5.62	7.89	24	72
2	2.0	12	11	L	0	45	111	86	206	6.47	745	5.62	7.89	24	72
2	3.0	12	11	L	0	70	138	88	207	12.59	726	6.99	8.58	2	10
2	3.0	12	11	L	0	70	134	85	206	12.59	726	6.99	8.58	2	10
2	3.0	12	11	L	0	71	133	87	203	12.59	726	6.99	8.58	2	10
2	3.0	12	11	L	0	69	149	90	200	13.6	718	7.54	8.71	2	10
2	3.0	12	11	L	0	72	120	86	210	13.6	718	7.54	8.71	2	10
2	3.0	12	11	L	0	67	150	92	204	13.6	718	7.54	8.71	2	10
2	1.0	19	11	L	0	67	137	94	201	13.94	577	8.60	8.32	41	61
2	1.0	19	11	L	0	64	132	93	217	13.94	577	8.60	8.32	41	61
2	1.0	19	11	L	0	70	134	92	207	13.94	577	8.60	8.32	41	61
2	2.0	19	11	L	0	46	72	89	198	6.72	742	4.32	7.73	26	98
2	2.0	19	11	L	0	42	88	86	196	6.72	742	4.32	7.73	26	98
2	2.0	19	11	L	0	46	83	86	188	6.72	742	4.32	7.73	26	98
2	3.0	19	11	L	0	115	205	96	197	19.59	569	11.46	8.34	2	10
2	3.0	19	11	L	0	109	216	92	208	19.59	569	11.46	8.34	2	10
2	3.0	19	11	L	0	119	218	96	213	19.59	569	11.46	8.34	2	10
2	3.0	19	11	L	0	136	241	110	216	18.14	602	10.64	8.45	2	10
2	3.0	19	11	L	0	135	260	98	227	18.14	602	10.64	8.45	2	10
2	3.0	19	11	L	0	133	253	102	228	18.14	602	10.64	8.45	2	10
2	1.0	26	11	L	0	56	117	92	214	12	558	6.71	8.39	41	60
2	1.0	26	11	L	0	57	120	95	210	12	558	6.71	8.39	41	60
2	1.0	26	11	L	0	57	134	90	213	12	558	6.71	8.39	41	60
2	2.0	26	11	L	0	45	83	86	190	6.52	750	4.82	7.64	26	98
2	2.0	26	11	L	0	46	90	96	200	6.52	750	4.82	7.64	26	98
2	2.0	26	11	L	0	45	88	90	194	6.52	750	4.82	7.64	26	98
2	3.0	26	11	L	0	107	206	93	203	13.33	617	8.80	8.37	2	12
2	3.0	26	11	L	0	107	218	96	202	13.33	617	8.80	8.37	2	12
2	3.0	26	11	L	0	102	208	94	237	13.33	617	8.80	8.37	2	12
2	3.0	26	11	L	0	107	199	96	235	15.01	595	8.92	8.39	2	12
2	3.0	26	11	L	0	105	210	100	239	15.01	595	8.92	8.39	2	12
2	3.0	26	11	L	0	104	223	103	236	15.01	595	8.92	8.39	2	12
3	1.0	1	11	L	0	68	197	95	240	10.4	615	5.54	7.91		
3	1.0	1	11	L	0	74	208	97	260	10.4	615	5.54	7.91		
3	1.0	1	11	L	0	73	208	95	259	10.4	615	5.54	7.91		

3	2.0	1	11	L	0	85	196	101	242	10.29	616	5.16	7.93		
3	2.0	1	11	L	0	82	206	103	254	10.29	616	5.16	7.93		
3	2.0	1	11	L	0	79	218	97	254	10.29	616	5.16	7.93		
3	3.0	1	11	L	0	77	191	98	244	9.59	617	3.68	8.08		
3	3.0	1	11	L	0	72	194	100	240	9.59	617	3.68	8.08		
3	3.0	1	11	L	0	74	196	101	245	9.59	617	3.68	8.08		
3	1.0	2	11	L	0	79	214	102	240	8.23	607	4.36	7.99		
3	1.0	2	11	L	0	81	201	100	251	8.23	607	4.36	7.99		
3	1.0	2	11	L	0	82	205	101	248	8.23	607	4.36	7.99		
3	2.0	2	11	L	0	88	201	105	248	7.92	609	4.20	7.95		
3	2.0	2	11	L	0	81	204	101	256	7.92	609	4.20	7.95		
3	2.0	2	11	L	0	89	197	99	254	7.92	609	4.20	7.95		
3	2.0	2	11	L	0	78	204	105	265	7.76	609	4.01	7.95		
3	2.0	2	11	L	0	74	196	103	254	7.76	609	4.01	7.95		
3	2.0	2	11	L	0	81	199	104	269	7.76	609	4.01	7.95		
3	3.0	2	11	L	0	78	195	97	264	6.1	601	3.48	8.23		
3	3.0	2	11	L	0	78	194	104	252	6.1	601	3.48	8.23		
3	3.0	2	11	L	0	80	206	99	247	6.1	601	3.48	8.23		
3	1.0	3	11	L	0	86	205	101	271	8.32	604	3.65	8.1		
3	1.0	3	11	L	0	86	205	103	263	8.32	604	3.65	8.1		
3	1.0	3	11	L	0	80	200	106	273	8.32	604	3.65	8.1		
3	2.0	3	11	L	0	75	193	100	242	7.76	609	3.07	7.96		
3	2.0	3	11	L	0	71	189	99	255	7.76	609	3.07	7.96		
3	2.0	3	11	L	0	81	199	108	258	7.76	609	3.07	7.96		
3	2.0	3	11	L	0	76	197	100	251	7.36	612	3.22	7.93		
3	2.0	3	11	L	0	74	206	105	253	7.36	612	3.22	7.93		
3	2.0	3	11	L	0	77	199	102	276	7.36	612	3.22	7.93		
3	3.0	3	11	L	0	69	205	103	274	6.22	604	3.55	8.21		
3	3.0	3	11	L	0	74	188	102	262	6.22	604	3.55	8.21		
3	3.0	3	11	L	0	74	205	102	275	6.22	604	3.55	8.21		
3	1.0	4	11	L	0	76	205	105	254	10.67	594	4.64	8.38		1
3	1.0	4	11	L	0	76	214	100	262	10.67	594	4.64	8.38		1
3	1.0	4	11	L	0	89	206	105	279	10.67	594	4.64	8.38		1
3	2.0	4	11	L	0	82	213	103	262	6.65	603	3.69	7.85		
3	2.0	4	11	L	0	81	212	109	275	6.65	603	3.69	7.85		
3	2.0	4	11	L	0	88	202	111	264	6.65	603	3.69	7.85		
3	2.0	4	11	L	0	72	189	102	275	7.58	596	3.27	7.96		
3	2.0	4	11	L	0	79	191	101	258	7.58	596	3.27	7.96		
3	2.0	4	11	L	0	80	194	107	256	7.58	596	3.27	7.96		
3	3.0	4	11	L	0	81	192	101	248	5.87	596	2.95	8.16		
3	3.0	4	11	L	0	75	185	101	251	5.87	596	2.95	8.16		
3	3.0	4	11	L	0	76	203	98	255	5.87	596	2.95	8.16		
3	1.0	5	11	L	0	91	223	103	261	16.09	584	5.02	8.99		10
3	1.0	5	11	L	0	90	228	110	281	16.09	584	5.02	8.99		10
3	1.0	5	11	L	0	89	234	108	275	16.09	584	5.02	8.99		10
3	2.0	5	11	L	0	74	190	104	258	8.32	602	3.54	8.17	4	
3	2.0	5	11	L	0	76	204	106	257	8.32	602	3.54	8.17	4	
3	2.0	5	11	L	0	76	189	106	262	8.32	602	3.54	8.17	4	
3	2.0	5	11	L	0	80	204	110	271	7.93	599	3.83	8.11	4	
3	2.0	5	11	L	0	74	200	107	271	7.93	599	3.83	8.11	4	
3	2.0	5	11	L	0	80	210	111	280	7.93	599	3.83	8.11	4	
3	3.0	5	11	L	0	77	189	106	262	7.19	603	2.75	8.42	2	3
3	3.0	5	11	L	0	76	181	106	271	7.19	603	2.75	8.42	2	3
3	3.0	5	11	L	0	80	190	106	276	7.19	603	2.75	8.42	2	3
3	1.0	12	11	L	0	98	246	106	280	21.45	590	6.75	8.38	14	103
3	1.0	12	11	L	0	90	246	106	303	21.45	590	6.75	8.38	14	103
3	1.0	12	11	L	0	94	250	105	361	21.45	590	6.75	8.38	14	103
3	2.0	12	11	L	0	92	232	109	286	6.47	745	6.27	7.89	48	107
3	2.0	12	11	L	0	92	246	104	297	6.47	745	6.27	7.89	48	107
3	2.0	12	11	L	0	88	235	107	284	6.47	745	6.27	7.89	48	107
3	2.0	12	11	L	0	94	238	102	282	12.59	726	7.70	8.58	48	107
3	2.0	12	11	L	0	94	242	102	278	12.59	726	7.70	8.58	48	107
3	2.0	12	11	L	0	98	232	104	301	12.59	726	7.70	8.58	48	107
3	3.0	12	11	L	0	88	230	103	268	13.6	718	7.00	8.71	8	13
3	3.0	12	11	L	0	89	228	106	280	13.6	718	7.00	8.71	8	13

3	3.0	12	11	L	0	86	230	113	270	13.6	718	7.00	8.71	8	13
3	1.0	19	11	L	0	119	303	113	363	13.94	577	9.63	8.32	14	113
3	1.0	19	11	L	0	109	309	111	350	13.94	577	9.63	8.32	14	113
3	1.0	19	11	L	0	110	297	116	340	13.94	577	9.63	8.32	14	113
3	2.0	19	11	L	0	110	286	108	340	6.72	742	12.49	7.73	48	107
3	2.0	19	11	L	0	113	300	108	362	6.72	742	12.49	7.73	48	107
3	2.0	19	11	L	0	114	302	111	313	6.72	742	12.49	7.73	48	107
3	2.0	19	11	L	0	104	273	108	319	19.59	569	8.00	8.34	48	107
3	2.0	19	11	L	0	104	286	108	322	19.59	569	8.00	8.34	48	107
3	2.0	19	11	L	0	106	269	113	318	19.59	569	8.00	8.34	48	107
3	3.0	19	11	L	0	120	270	108	294	18.14	602	12.99	8.45	9	13
3	3.0	19	11	L	0	117	275	107	295	18.14	602	12.99	8.45	9	13
3	3.0	19	11	L	0	111	286	105	291	18.14	602	12.99	8.45	9	13
3	1.0	26	11	L	0	101	287	113	381	12	558	8.89	8.39	14	114
3	1.0	26	11	L	0	104	278	114	361	12	558	8.89	8.39	14	114
3	1.0	26	11	L	0	101	294	114	384	12	558	8.89	8.39	14	114
3	2.0	26	11	L	0	117	295	110	353	6.52	750	9.73	7.64	48	112
3	2.0	26	11	L	0	114	315	114	349	6.52	750	9.73	7.64	48	112
3	2.0	26	11	L	0	114	303	110	403	6.52	750	9.73	7.64	48	112
3	2.0	26	11	L	0	110	327	110	339	13.33	617	10.20	8.37	48	112
3	2.0	26	11	L	0	111	322	106	295	13.33	617	10.20	8.37	48	112
3	2.0	26	11	L	0	110	338	106	301	13.33	617	10.20	8.37	48	112
3	3.0	26	11	L	0	107	286	106	284	15.01	595	10.83	8.39	9	17
3	3.0	26	11	L	0	109	279	103	304	15.01	595	10.83	8.39	9	17
3	3.0	26	11	L	0	111	285	103	291	15.01	595	10.83	8.39	9	17
4	1.0	1	11	L	4	76	249	91	249	9.28	951	18.10	7.69		
4	1.0	1	11	L	4	70	233	101	252	9.28	951	18.10	7.69		
4	1.0	1	11	L	4	79	234	98	261	9.28	951	18.10	7.69		
4	2.0	1	11	L	4	70	250	94	257	9.28	951	13.71	7.69		
4	2.0	1	11	L	4	79	269	98	265	9.28	951	13.71	7.69		
4	2.0	1	11	L	4	79	241	97	261	9.28	951	13.71	7.69		
4	3.0	1	11	L	4	68	231	99	261	9.28	951	10.10	7.69		
4	3.0	1	11	L	4	74	249	98	260	9.28	951	10.10	7.69		
4	3.0	1	11	L	4	71	237	102	269	9.28	951	10.10	7.69		
4	1.0	2	11	L	4	88	242	98	253	9.46	925	10.13	8.1		
4	1.0	2	11	L	4	80	227	97	252	9.46	925	10.13	8.1		
4	1.0	2	11	L	4	74	235	98	268	9.46	925	10.13	8.1		
4	1.0	2	11	L	4	83	217	88	245	9.74	911	10.84	8.12		
4	1.0	2	11	L	4	84	235	93	235	9.74	911	10.84	8.12		
4	1.0	2	11	L	4	83	226	91	226	9.74	911	10.84	8.12		
4	2.0	2	11	L	4	85	231	101	252	7.55	934	8.27	7.87		
4	2.0	2	11	L	4	75	231	97	264	7.55	934	8.27	7.87		
4	2.0	2	11	L	4	80	236	95	265	7.55	934	8.27	7.87		
4	3.0	2	11	L	4										
4	3.0	2	11	L	4										
4	3.0	2	11	L	4										
4	1.0	3	11	L	4	77	218	93	244	13.89	908	11.62	8.63		
4	1.0	3	11	L	4	82	225	87	244	13.89	908	11.62	8.63		
4	1.0	3	11	L	4	74	235	91	260	13.89	908	11.62	8.63		
4	1.0	3	11	L	4	77	232	88	232	13.99	898	11.52	8.66		
4	1.0	3	11	L	4	75	231	90	244	13.99	898	11.52	8.66		
4	1.0	3	11	L	4	77	222	92	231	13.99	898	11.52	8.66		
4	2.0	3	11	L	4	80	219	92	235	7.62	921	9.63	7.91		
4	2.0	3	11	L	4	85	228	90	252	7.62	921	9.63	7.91		
4	2.0	3	11	L	4	72	222	94	244	7.62	921	9.63	7.91		
4	3.0	3	11	L	4										
4	3.0	3	11	L	4										
4	3.0	3	11	L	4										
4	1.0	4	11	L	4	85	221	88	232	15.9	897	11.27	9.11	8	11
4	1.0	4	11	L	4	84	234	92	240	15.9	897	11.27	9.11	8	11
4	1.0	4	11	L	4	87	247	91	247	15.9	897	11.27	9.11	8	11
4	1.0	4	11	L	4	93	233	94	227	15.68	890	10.67	9.15	8	11
4	1.0	4	11	L	4	83	226	85	244	15.68	890	10.67	9.15	8	11
4	1.0	4	11	L	4	84	239	90	238	15.68	890	10.67	9.15	8	11
4	2.0	4	11	L	4	80	220	91	248	6.85	925	8.68	7.92		

4	2.0	4	11	L	4	81	225	95	240	6.85	925	8.68	7.92		
4	2.0	4	11	L	4	81	225	81	260	6.85	925	8.68	7.92		
4	3.0	4	11	L	4										
4	3.0	4	11	L	4										
4	3.0	4	11	L	4										
4	1.0	5	11	L	4	74	226	88	238	18.98	882	1.99	9.24	18	40
4	1.0	5	11	L	4	81	218	90	249	18.98	882	1.99	9.24	18	40
4	1.0	5	11	L	4	81	231	93	242	18.98	882	1.99	9.24	18	40
4	1.0	5	11	L	4	79	241	87	237	18.85	882	2.01	9.26	18	40
4	1.0	5	11	L	4	84	224	90	245	18.85	882	2.01	9.26	18	40
4	1.0	5	11	L	4	82	224	93	255	18.85	882	2.01	9.26	18	40
4	2.0	5	11	L	4	79	220	93	236	7.42	923		7.97	5	15
4	2.0	5	11	L	4	83	229	92	246	7.42	923		7.97	5	15
4	2.0	5	11	L	4	82	215	91	256	7.42	923		7.97	5	15
4	3.0	5	11	L	4	83	227	89	233	7.18	926		8.6	1	3
4	3.0	5	11	L	4	81	217	91	260	7.18	926		8.6	1	3
4	3.0	5	11	L	4	74	211	95	250	7.18	926		8.6	1	3
4	1.0	12	11	L	4	82	240	91	242	28.08	812	12.25	10.16	161	212
4	1.0	12	11	L	4	84	235	90	242	28.08	812	12.25	10.16	161	212
4	1.0	12	11	L	4	83	242	91	251	28.08	812	12.25	10.16	161	212
4	1.0	12	11	L	4	82	232	92	242	28.08	812	12.25	10.16	161	212
4	1.0	12	11	L	4	83	235	88	236	26.23	823	11.52	10.18	161	212
4	1.0	12	11	L	4	83	241	87	257	26.23	823	11.52	10.18	161	212
4	2.0	12	11	L	4	78	218	92	239	12.5	884	10.66	8.54	43	252
4	2.0	12	11	L	4	81	215	92	252	12.5	884	10.66	8.54	43	252
4	2.0	12	11	L	4	80	229	91	252	12.5	884	10.66	8.54	43	252
4	3.0	12	11	L	4	77	221	91	242	12.65	890	7.94	9.15	6	15
4	3.0	12	11	L	4	77	211	85	234	12.65	890	7.94	9.15	6	15
4	3.0	12	11	L	4	81	212	89	247	12.65	890	7.94	9.15	6	15
4	1.0	19	11	L	4	91	241	127	369	30.76	901	17.29	11.5	164	287
4	1.0	19	11	L	4	89	272	129	390	30.76	901	17.29	11.5	164	287
4	1.0	19	11	L	4	93	240	129	416	30.76	901	17.29	11.5	164	287
4	1.0	19	11	L	4	91	264	126	492	31.56	894	18.81	11.53	164	287
4	1.0	19	11	L	4	91	225	135	483	31.56	894	18.81	11.53	164	287
4	1.0	19	11	L	4	90	244	132	434	31.56	894	18.81	11.53	164	287
4	2.0	19	11	L	4	71	208	90	232	15.61	906	8.75	9.03	43	324
4	2.0	19	11	L	4	83	206	93	232	15.61	906	8.75	9.03	43	324
4	2.0	19	11	L	4	71	203	84	231	15.61	906	8.75	9.03	43	324
4	3.0	19	11	L	4	82	225	84	231	20.46	845	10.56	9.79	7	25
4	3.0	19	11	L	4	81	236	87	238	20.46	845	10.56	9.79	7	25
4	3.0	19	11	L	4	80	246	84	230	20.46	845	10.56	9.79	7	25
4	1.0	26	11	L	4	115	250	128	507	14.72	856	20.25	11.43	165	306
4	1.0	26	11	L	4	106	252	135	433	14.72	856	20.25	11.43	165	306
4	1.0	26	11	L	4	108	261	130	442	14.72	856	20.25	11.43	165	306
4	1.0	26	11	L	4	109	292	136	510	17.3	881	18.17	11.5	165	306
4	1.0	26	11	L	4	111	302	139	460	17.3	881	18.17	11.5	165	306
4	1.0	26	11	L	4	109	289	139	533	17.3	881	18.17	11.5	165	306
4	2.0	26	11	L	4	84	237	89	262	23.3	828	11.36	10.25	43	341
4	2.0	26	11	L	4	86	246	87	243	23.3	828	11.36	10.25	43	341
4	2.0	26	11	L	4	82	245	92	255	23.3	828	11.36	10.25	43	341
4	3.0	26	11	L	4	81	235	80	234	30.21	873	14.43	11.47	7	25
4	3.0	26	11	L	4	83	231	82	233	30.21	873	14.43	11.47	7	25
4	3.0	26	11	L	4	86	233	80	235	30.21	873	14.43	11.47	7	25
5	1.0	1	11	L	2	84	351	164	431	8.47	537	3.89	7.83		
5	1.0	1	11	L	2	100	318	187	462	8.47	537	3.89	7.83		
5	1.0	1	11	L	2	82	323	167	457	8.47	537	3.89	7.83		
5	2.0	1	11	L	2	90	236	164	424	8.47	537	5.16	7.83		
5	2.0	1	11	L	2	102	247	166	426	8.47	537	5.16	7.83		
5	2.0	1	11	L	2	85	297	164	456	8.47	537	5.16	7.83		
5	3.0	1	11	L	2	74	249	164	424	8.47	537	4.56	7.83		
5	3.0	1	11	L	2	72	316	164	426	8.47	537	4.56	7.83		
5	3.0	1	11	L	2	102	244	164	531	8.47	537	4.56	7.83		
5	1.0	2	11	L	2	85	321	164	407	7.67	526	2.83	7.78		
5	1.0	2	11	L	2	85	316	158	404	7.67	526	2.83	7.78		
5	1.0	2	11	L	2	86	329	161	419	7.67	526	2.83	7.78		

5	2.0	2	11	L	2	78	321	161	415	8.2	531	5.11	7.94		
5	2.0	2	11	L	2	81	311	156	435	8.2	531	5.11	7.94		
5	2.0	2	11	L	2	83	329	153	417	8.2	531	5.11	7.94		
5	2.0	2	11	L	2	88	319	151	434	8.07	528	4.27	7.76		
5	2.0	2	11	L	2	85	324	159	419	8.07	528	4.27	7.76		
5	2.0	2	11	L	2	78	334	156	465	8.07	528	4.27	7.76		
5	3.0	2	11	L	2										
5	3.0	2	11	L	2										
5	3.0	2	11	L	2										
5	1.0	3	11	L	2	82	343	148	417	7.06	533	4.61	7.71		
5	1.0	3	11	L	2	91	312	150	403	7.06	533	4.61	7.71		
5	1.0	3	11	L	2	99	340	160	414	7.06	533	4.61	7.71		
5	2.0	3	11	L	2	91	313	162	414	7.32	531	5.18	7.63		
5	2.0	3	11	L	2	84	363	160	422	7.32	531	5.18	7.63		
5	2.0	3	11	L	2	97	304	167	434	7.41	531	4.83	7.77		
5	2.0	3	11	L	2	84	328	152	431	7.41	531	4.83	7.77		
5	2.0	3	11	L	2	101	332	158	432	7.41	531	4.83	7.77		
5	2.0	3	11	L	2	94	321	159	432	7.41	531	4.83	7.77		
5	3.0	3	11	L	2										
5	3.0	3	11	L	2										
5	3.0	3	11	L	2										
5	1.0	4	11	L	2	101	326	150	412	6.5	530	2.90	7.62	90	208
5	1.0	4	11	L	2	102	303	163	420	6.5	530	2.90	7.62	90	208
5	1.0	4	11	L	2	79	310	161	445	6.5	530	2.90	7.62	90	208
5	2.0	4	11	L	2	104	354	155	440	6.92	527	3.10	7.7		
5	2.0	4	11	L	2	89	336	160	433	6.92	527	3.10	7.7		
5	2.0	4	11	L	2	109	358	157	417	6.92	527	3.10	7.7		
5	2.0	4	11	L	2	129	363	153	404	7.07	527	3.54	7.75		
5	2.0	4	11	L	2	105	366	160	428	7.07	527	3.54	7.75		
5	2.0	4	11	L	2	105	343	166	425	7.07	527	3.54	7.75		
5	3.0	4	11	L	2										
5	3.0	4	11	L	2										
5	3.0	4	11	L	2										
5	1.0	5	11	L	2	98	271	158	406	6.98	531	3.38	7.63	154	288
5	1.0	5	11	L	2	98	269	158	429	6.98	531	3.38	7.63	154	288
5	1.0	5	11	L	2	84	295	172	466	6.98	531	3.38	7.63	154	288
5	2.0	5	11	L	2	96	325	158	451	7.18	528	3.14	7.74	1	8
5	2.0	5	11	L	2	88	321	158	422	7.18	528	3.14	7.74	1	8
5	2.0	5	11	L	2	93	358	167	439	7.18	528	3.14	7.74	1	8
5	2.0	5	11	L	2	86	374	168	446	7.37	516	2.54	7.78		
5	2.0	5	11	L	2	94	382	160	426	7.37	516	2.54	7.78		
5	2.0	5	11	L	2	93	353	160	424	7.37	516	2.54	7.78		
5	3.0	5	11	L	2	84	347	146	434	7.05	525	2.93	7.82		
5	3.0	5	11	L	2	86	320	150	433	7.05	525	2.93	7.82		
5	3.0	5	11	L	2	86	315	163	422	7.05	525	2.93	7.82		
5	1.0	12	11	L	2	139	441	182	429	24.45	380	9.72	10.46	191	>300
5	1.0	12	11	L	2	134	441	172	464	24.45	380	9.72	10.46	191	>300
5	1.0	12	11	L	2	149	441	184	481	24.45	380	9.72	10.46	191	>300
5	2.0	12	11	L	2	75	301	155	427	6.39	534	3.22	7.51	16	57
5	2.0	12	11	L	2	73	327	159	399	6.39	534	3.22	7.51	16	57
5	2.0	12	11	L	2	68	280	162	434	6.39	534	3.22	7.51	16	57
5	2.0	12	11	L	2	70	267	157	459	6.62	538	3.27	7.5	16	57
5	2.0	12	11	L	2	72	262	157	439	6.62	538	3.27	7.5	16	57
5	2.0	12	11	L	2	73	285	162	437	6.62	538	3.27	7.5	16	57
5	3.0	12	11	L	2	83	377	154	429	6.8	537	3.74	7.73	2	8
5	3.0	12	11	L	2	92	367	152	421	6.8	537	3.74	7.73	2	8
5	3.0	12	11	L	2	90	329	159	444	6.8	537	3.74	7.73	2	8
5	1.0	19	11	L	2	112	400	168	527	18.09	446	9.54	11.34	198	>300
5	1.0	19	11	L	2	104	420	170	498	18.09	446	9.54	11.34	198	>300
5	1.0	19	11	L	2	107	405	183	675	18.09	446	9.54	11.34	198	>300
5	2.0	19	11	L	2	92	262	158	417	8.35	536	4.15	7.54	17	69
5	2.0	19	11	L	2	94	255	165	435	8.35	536	4.15	7.54	17	69
5	2.0	19	11	L	2	96	259	163	446	8.35	536	4.15	7.54	17	69
5	2.0	19	11	L	2	101	249	157	422	8.39	535	4.21	7.55	17	69
5	2.0	19	11	L	2	89	269	161	445	8.39	535	4.21	7.55	17	69

5	2.0	19	11	L	2	89	245	160	430	8.39	535	4.21	7.55	17	69
5	3.0	19	11	L	2	115	305	155	428	8.31	526	5.19	7.77	2	9
5	3.0	19	11	L	2	110	288	155	453	8.31	526	5.19	7.77	2	9
5	3.0	19	11	L	2	97	295	161	450	8.31	526	5.19	7.77	2	9
5	1.0	26	11	L	2	131	423	169	475	13.35	470	11.34	11.37	206	>300
5	1.0	26	11	L	2	138	418	169	509	13.35	470	11.34	11.37	206	>300
5	1.0	26	11	L	2	144	444	169	528	13.35	470	11.34	11.37	206	>300
5	2.0	26	11	L	2	70	258	154	412	8.74	542	3.54	7.47	17	80
5	2.0	26	11	L	2	70	258	159	407	8.74	542	3.54	7.47	17	80
5	2.0	26	11	L	2	104	251	156	402	8.74	542	3.54	7.47	17	80
5	2.0	26	11	L	2	68	253	157	400	8.56	539	3.43	7.45	17	80
5	2.0	26	11	L	2	73	253	157	439	8.56	539	3.43	7.45	17	80
5	2.0	26	11	L	2	75	246	156	413	8.56	539	3.43	7.45	17	80
5	3.0	26	11	L	2	76	287	154	467	8.68	531	3.78	7.68	2	12
5	3.0	26	11	L	2	73	288	152	444	8.68	531	3.78	7.68	2	12
5	3.0	26	11	L	2	81	288	160	405	8.68	531	3.78	7.68	2	12
6	3.0	1	11	L	1	3	31	3	8			0.33			
6	3.0	1	11	L	1	3	31	3	8			0.33			
6	3.0	1	11	L	1	3	31	3	8			0.33			
6	3.0	1	11	L	1	3	31	3	8			0.33			
6	1.0	1	11	L	1	2	22	2	7			0.18			
6	1.0	1	11	L	1	2	22	2	7			0.18			
6	1.0	1	11	L	1	2	22	2	7			0.18			
6	1.0	1	11	L	1	2	22	2	7			0.18			
6	2.0	1	11	L	1	1	23	3	5			0.34			
6	2.0	1	11	L	1	1	23	3	5			0.34			
6	2.0	1	11	L	1	1	23	3	5			0.34			
6	2.0	1	11	L	1	1	23	3	5			0.34			
6	3.0	2	11	L	1	4	40	3	37						
6	1.0	2	11	L	1	3	16	3	26						
6	2.0	2	11	L	1	3	41	4	39						
6	3.0	4	11	L	1	3	39	4	61			0.36			
6	1.0	4	11	L	1	3	39	3	46			0.21			
6	2.0	4	11	L	1	4	26	5	89			0.21			
6	3.0	5	11	L	1	4	31	4	49			0.45			
6	1.0	5	11	L	1	3	19	3	27			0.26			
6	2.0	5	11	L	1	3	22	4	48			0.30			
6	3.0	12	11	L	1	4	36	3	57			0.61			
6	1.0	12	11	L	1	4	50	6	69			0.44		1	
6	2.0	12	11	L	1	5	38	6	58			0.53			
6	3.0	19	11	L	1	5	53	6	86			0.81			
6	1.0	19	11	L	1	5	36	4	58			0.58		1	
6	2.0	19	11	L	1	3	27	6	88			0.74		1	
6	3.0	26	11	L	1	7	42	5	81			1.22			
6	1.0	26	11	L	1	5	23	6	70			0.80		1	
6	2.0	26	11	L	1	4	35	4	82			0.73		1	
2	1.0	1	11	D	0	44	130	78	172	10.58	734	4.86	7.82		
2	1.0	1	11	D	0	47	118	81	181	10.58	734	4.86	7.82		
2	1.0	1	11	D	0	43	119	80	178	10.58	734	4.86	7.82		
2	2.0	1	11	D	0	42	124	81	174	10.58	738		7.9		
2	2.0	1	11	D	0	56	133	86	175	10.58	738		7.9		
2	2.0	1	11	D	0	55	128	80	184	10.58	738		7.9		
2	3.0	1	11	D	0	37	125	75	160	10.58	724		8.07		
2	3.0	1	11	D	0	48	125	75	182	10.58	724		8.07		
2	3.0	1	11	D	0	48	133	75	170	10.58	724		8.07		
2	1.0	2	11	D	0	52	139	82	193	8.18	742	4.70	8		
2	1.0	2	11	D	0	57	141	88	196	8.18	742	4.70	8		
2	1.0	2	11	D	0	51	129	84	198	8.18	742	4.70	8		
2	2.0	2	11	D	0	46	137	84	198	8.11	736	4.67	8.04		
2	2.0	2	11	D	0	46	120	86	200	8.11	736	4.67	8.04		
2	2.0	2	11	D	0	50	149	86	194	8.11	736	4.67	8.04		
2	3.0	2	11	D	0	50	119	79	176	6.8	734	4.23	8.13		
2	3.0	2	11	D	0	50	127	81	180	6.8	734	4.23	8.13		
2	3.0	2	11	D	0	45	117	84	182	6.8	734	4.23	8.13		
2	3.0	2	11	D	0	46	126	80	184	6.83	738	4.39	8.19		

2	3.0	2	11	D	0	49	127	80	191	6.83	738	4.39	8.19		
2	3.0	2	11	D	0	46	123	79	183	6.83	738	4.39	8.19		
2	1.0	3	11	D	0	48	132	83	180	8.11	725		8.03		
2	1.0	3	11	D	0	47	113	85	179	8.11	725		8.03		
2	1.0	3	11	D	0	52	128	84	177	8.11	725		8.03		
2	2.0	3	11	D	0	50	115	78	175	8.12	736		8.06		
2	2.0	3	11	D	0	55	126	85	179	8.12	736		8.06		
2	2.0	3	11	D	0	54	116	81	171	8.12	736		8.06		
2	3.0	3	11	D	0	51	128	84	168	7.42	727		8.22		
2	3.0	3	11	D	0	53	119	77	179	7.42	727		8.22		
2	3.0	3	11	D	0	51	116	82	187	7.42	727		8.22		
2	3.0	3	11	D	0	44	119	77	171	7.28	726		8.22		
2	3.0	3	11	D	0	46	119	75	172	7.28	726		8.22		
2	3.0	3	11	D	0	47	119	78	174	7.28	726		8.22		
2	1.0	4	11	D	0	44	112	81	193	8.6	740	1.76	8.08	6	
2	1.0	4	11	D	0	51	109	83	181	8.6	740	1.76	8.08	6	
2	1.0	4	11	D	0	53	121	85	179	8.6	740	1.76	8.08	6	
2	2.0	4	11	D	0	46	118	77	186	8.52	737	1.50	8.09		
2	2.0	4	11	D	0	49	117	89	183	8.52	737	1.50	8.09		
2	2.0	4	11	D	0	53	120	80	191	8.52	737	1.50	8.09		
2	3.0	4	11	D	0	49	121	75	174	7.73	733	3.35	8.26		
2	3.0	4	11	D	0	45	130	76	195	7.73	733	3.35	8.26		
2	3.0	4	11	D	0	48	113	80	176	7.73	733	3.35	8.26		
2	3.0	4	11	D	0	45	124	85	173	7.67	738	1.53	8.27		
2	3.0	4	11	D	0	42	117	79	177	7.67	738	1.53	8.27		
2	3.0	4	11	D	0	42	121	80	180	7.67	738	1.53	8.27		
2	1.0	5	11	D	0	45	115	84	197	8.01	741	5.06	8.01		23
2	1.0	5	11	D	0	42	108	82	179	8.01	741	5.06	8.01		23
2	1.0	5	11	D	0	45	114	85	179	8.01	741	5.06	8.01		23
2	2.0	5	11	D	0	50	114	79	188	8.03	742	5.11	8.05		5
2	2.0	5	11	D	0	44	120	81	174	8.03	742	5.11	8.05		5
2	2.0	5	11	D	0	60	113	81	186	8.03	742	5.11	8.05		5
2	3.0	5	11	D	0	48	125	80	174	6.89	741	4.29	8.2		2
2	3.0	5	11	D	0	48	128	80	180	6.89	741	4.29	8.2		2
2	3.0	5	11	D	0	45	138	82	189	6.89	741	4.29	8.2		2
2	3.0	5	11	D	0	42	120	76	183	7.4	743	4.70	8.19		2
2	3.0	5	11	D	0	50	122	83	179	7.4	743	4.70	8.19		2
2	3.0	5	11	D	0	46	117	83	177	7.4	743	4.70	8.19		2
2	1.0	12	11	D	0	49	91	84	197	7.87	744	3.72	7.9	82	93
2	1.0	12	11	D	0	42	100	90	197	7.87	744	3.72	7.9	82	93
2	1.0	12	11	D	0	45	85	89	201	7.87	744	3.72	7.9	82	93
2	2.0	12	11	D	0	48	90	90	203	7.75	747	4.06	7.92	41	32
2	2.0	12	11	D	0	41	90	85	214	7.75	747	4.06	7.92	41	32
2	2.0	12	11	D	0	49	85	89	199	7.75	747	4.06	7.92	41	32
2	3.0	12	11	D	0	44	115	87	193	7.43	741	5.25	8.07	3	5
2	3.0	12	11	D	0	37	95	90	207	7.43	741	5.25	8.07	3	5
2	3.0	12	11	D	0	46	115	85	197	7.43	741	5.25	8.07	3	5
2	3.0	12	11	D	0	50	110	85	189	7.2	747	4.51	8.07	3	5
2	3.0	12	11	D	0	41	119	85	202	7.2	747	4.51	8.07	3	5
2	3.0	12	11	D	0	51	116	87	208	7.2	747	4.51	8.07	3	5
2	1.0	19	11	D	0	43	60	89	212	7.84	746	4.18	7.81	73	93
2	1.0	19	11	D	0	46	68	88	211	7.84	746	4.18	7.81	73	93
2	1.0	19	11	D	0	44	79	87	198	7.84	746	4.18	7.81	73	93
2	2.0	19	11	D	0	45	79	89	208	8.12	748	3.68	7.91	37	27
2	2.0	19	11	D	0	45	97	95	207	8.12	748	3.68	7.91	37	27
2	2.0	19	11	D	0	42	76	95	206	8.12	748	3.68	7.91	37	27
2	3.0	19	11	D	0	49	124	90	205	7.95	744	4.16	8.09	3	8
2	3.0	19	11	D	0	53	125	87	220	7.95	744	4.16	8.09	3	8
2	3.0	19	11	D	0	49	132	88	205	7.95	744	4.16	8.09	3	8
2	3.0	19	11	D	0	45	110	88	195	7.91	746	4.13	8.03	3	8
2	3.0	19	11	D	0	46	120	92	203	7.91	746	4.13	8.03	3	8
2	3.0	19	11	D	0	50	103	88	202	7.91	746	4.13	8.03	3	8
2	1.0	26	11	D	0	41	63	92	202	7.73	749	4.26	7.85	77	110
2	1.0	26	11	D	0	44	65	88	219	7.73	749	4.26	7.85	77	110
2	1.0	26	11	D	0	45	69	87	201	7.73	749	4.26	7.85	77	110

2	2.0	26	11	D	0	45	83	92	198	7.7	748	4.84	7.88	28	27
2	2.0	26	11	D	0	51	88	93	216	7.7	748	4.84	7.88	28	27
2	2.0	26	11	D	0	42	84	91	236	7.7	748	4.84	7.88	28	27
2	3.0	26	11	D	0	41	65	90	207	7.71	737	4.22	8.06	3	7
2	3.0	26	11	D	0	37	83	94	223	7.71	737	4.22	8.06	3	7
2	3.0	26	11	D	0	38	83	92	217	7.71	737	4.22	8.06	3	7
2	3.0	26	11	D	0	46	83	88	200	7.78	744	4.56	8.08	3	7
2	3.0	26	11	D	0	41	92	91	197	7.78	744	4.56	8.08	3	7
2	3.0	26	11	D	0	46	82	88	200	7.78	744	4.56	8.08	3	7
3	1.0	1	11	D	0	68	197	95	240	10.4	615	5.54	7.91		
3	1.0	1	11	D	0	74	208	97	260	10.4	615	5.54	7.91		
3	1.0	1	11	D	0	73	208	95	259	10.4	615	5.54	7.91		
3	2.0	1	11	D	0	85	196	101	242	10.29	616	5.16	7.93		
3	2.0	1	11	D	0	82	206	103	254	10.29	616	5.16	7.93		
3	2.0	1	11	D	0	79	218	97	254	10.29	616	5.16	7.93		
3	3.0	1	11	D	0	77	191	98	244	9.59	617	3.68	8.08		
3	3.0	1	11	D	0	72	194	100	240	9.59	617	3.68	8.08		
3	3.0	1	11	D	0	74	196	101	245	9.59	617	3.68	8.08		
3	1.0	2	11	D	0	71	190	101	263	8.03	609	3.95	7.92		
3	1.0	2	11	D	0	78	197	104	265	8.03	609	3.95	7.92		
3	1.0	2	11	D	0	78	214	107	268	8.03	609	3.95	7.92		
3	2.0	2	11	D	0	81	184	98	248	7.79	603	4.37	7.96		
3	2.0	2	11	D	0	82	202	100	252	7.79	603	4.37	7.96		
3	2.0	2	11	D	0	75	206	101	251	7.79	603	4.37	7.96		
3	2.0	2	11	D	0	91	204	104	288	7.82	606	3.28	7.97		
3	2.0	2	11	D	0	87	192	107	289	7.82	606	3.28	7.97		
3	2.0	2	11	D	0	78	194	108	290	7.82	606	3.28	7.97		
3	3.0	2	11	D	0	74	189	104	244	6.77	604	3.54	8.18		
3	3.0	2	11	D	0	70	176	109	265	6.77	604	3.54	8.18		
3	3.0	2	11	D	0	82	190	100	252	6.77	604	3.54	8.18		
3	1.0	3	11	D	0	77	225	104	258	7.1	610	3.20	7.82		
3	1.0	3	11	D	0	79	206	104	269	7.1	610	3.20	7.82		
3	1.0	3	11	D	0	74	211	106	255	7.1	610	3.20	7.82		
3	2.0	3	11	D	0	77	193	102	257	7.67	613	3.27	7.99		
3	2.0	3	11	D	0	74	192	104	265	7.67	613	3.27	7.99		
3	2.0	3	11	D	0	78	187	104	273	7.67	613	3.27	7.99		
3	2.0	3	11	D	0	76	191	104	266	7.67	610	3.01	7.98		
3	2.0	3	11	D	0	83	203	105	261	7.67	610	3.01	7.98		
3	2.0	3	11	D	0	75	207	106	275	7.67	610	3.01	7.98		
3	3.0	3	11	D	0	74	178	98	257	6.53	606	2.99	8.2		
3	3.0	3	11	D	0	78	194	104	269	6.53	606	2.99	8.2		
3	3.0	3	11	D	0	78	180	104	261	6.53	606	2.99	8.2		
3	1.0	4	11	D	0	82	195	108	265	6.84	599	3.73	7.83		
3	1.0	4	11	D	0	76	193	102	281	6.84	599	3.73	7.83		
3	1.0	4	11	D	0	76	213	113	288	6.84	599	3.73	7.83		
3	2.0	4	11	D	0	70	192	111	254	7.64	599	3.25	7.94		
3	2.0	4	11	D	0	68	200	105	259	7.64	599	3.25	7.94		
3	2.0	4	11	D	0	81	204	100	258	7.64	599	3.25	7.94		
3	2.0	4	11	D	0	76	201	106	260	7.4	604	3.61	7.94		
3	2.0	4	11	D	0	80	209	108	260	7.4	604	3.61	7.94		
3	2.0	4	11	D	0	84	192	101	255	7.4	604	3.61	7.94		
3	3.0	4	11	D	0	72	189	100	249	6.3	604	2.85	8.12		
3	3.0	4	11	D	0	73	185	101	249	6.3	604	2.85	8.12		
3	3.0	4	11	D	0	85	186	105	258	6.3	604	2.85	8.12		
3	1.0	5	11	D	0	82	189	106	262	7.98	601	4.11	8.06	4	7
3	1.0	5	11	D	0	70	190	103	269	7.98	601	4.11	8.06	4	7
3	1.0	5	11	D	0	80	187	108	281	7.98	601	4.11	8.06	4	7
3	2.0	5	11	D	0	82	202	105	254	7.6	601	3.66	8.1		8
3	2.0	5	11	D	0	80	197	106	251	7.6	601	3.66	8.1		8
3	2.0	5	11	D	0	85	210	106	262	7.6	601	3.66	8.1		8
3	2.0	5	11	D	0	76	201	105	244	8.46	602	3.41	8.18		8
3	2.0	5	11	D	0	77	188	102	268	8.46	602	3.41	8.18		8
3	2.0	5	11	D	0	78	197	102	265	8.46	602	3.41	8.18		8
3	3.0	5	11	D	0	75	194	101	258	7.33	599	2.71	8.43		4
3	3.0	5	11	D	0	80	194	101	254	7.33	599	2.71	8.43		4

3	3.0	5	11	D	0	76	193	102	258	7.33	599	2.71	8.43		4
3	1.0	12	11	D	0	78	164	104	260	7.87	744	4.69	7.9	9	29
3	1.0	12	11	D	0	73	184	107	278	7.87	744	4.69	7.9	9	29
3	1.0	12	11	D	0	79	189	107	275	7.87	744	4.69	7.9	9	29
3	2.0	12	11	D	0	80	192	102	270	7.75	747	5.09	7.92	2	18
3	2.0	12	11	D	0	76	190	111	259	7.75	747	5.09	7.92	2	18
3	2.0	12	11	D	0	78	197	113	259	7.75	747	5.09	7.92	2	18
3	2.0	12	11	D	0	78	183	106	255	7.43	741	4.54	8.07	2	18
3	2.0	12	11	D	0	75	194	111	266	7.43	741	4.54	8.07	2	18
3	2.0	12	11	D	0	78	186	106	272	7.43	741	4.54	8.07	2	18
3	3.0	12	11	D	0	80	191	105	272	7.2	747	4.88	8.07		7
3	3.0	12	11	D	0	74	194	107	274	7.2	747	4.88	8.07		7
3	3.0	12	11	D	0	81	194	110	270	7.2	747	4.88	8.07		7
3	1.0	19	11	D	0	77	171	107	282	7.84	746	1.99	7.81	9	38
3	1.0	19	11	D	0	70	178	109	287	7.84	746	1.99	7.81	9	38
3	1.0	19	11	D	0	82	196	110	272	7.84	746	1.99	7.81	9	38
3	2.0	19	11	D	0	76	185	111	299	8.12	748	3.34	7.91	6	18
3	2.0	19	11	D	0	77	195	111	308	8.12	748	3.34	7.91	6	18
3	2.0	19	11	D	0	78	190	114	309	8.12	748	3.34	7.91	6	18
3	2.0	19	11	D	0	78	194	116	306	7.95	744	3.43	8.09	6	18
3	2.0	19	11	D	0	71	205	114	314	7.95	744	3.43	8.09	6	18
3	2.0	19	11	D	0	73	191	125	309	7.95	744	3.43	8.09	6	18
3	3.0	19	11	D	0	70	182	110	279	7.91	746	3.09	8.03		7
3	3.0	19	11	D	0	77	198	112	288	7.91	746	3.09	8.03		7
3	3.0	19	11	D	0	72	188	110	284	7.91	746	3.09	8.03		7
3	1.0	26	11	D	0	76	192	105	269	7.73	749	2.83	7.85	10	38
3	1.0	26	11	D	0	76	182	111	267	7.73	749	2.83	7.85	10	38
3	1.0	26	11	D	0	73	185	108	273	7.73	749	2.83	7.85	10	38
3	2.0	26	11	D	0	79	191	110	284	7.7	748	3.15	7.88	6	20
3	2.0	26	11	D	0	72	188	117	281	7.7	748	3.15	7.88	6	20
3	2.0	26	11	D	0	79	188	114	285	7.7	748	3.15	7.88	6	20
3	2.0	26	11	D	0	79	176	110	275	7.71	737	2.65	8.06	6	20
3	2.0	26	11	D	0	79	186	106	288	7.71	737	2.65	8.06	6	20
3	2.0	26	11	D	0	76	183	106	279	7.71	737	2.65	8.06	6	20
3	3.0	26	11	D	0	72	179	103	304	7.78	744	3.16	8.08		7
3	3.0	26	11	D	0	76	191	107	285	7.78	744	3.16	8.08		7
3	3.0	26	11	D	0	79	191	110	302	7.78	744	3.16	8.08		7
4	1.0	1	11	D	4	76	249	91	249	9.28	951	18.10	7.69		
4	1.0	1	11	D	4	70	233	101	252	9.28	951	18.10	7.69		
4	1.0	1	11	D	4	79	234	98	261	9.28	951	18.10	7.69		
4	2.0	1	11	D	4	70	250	94	257	9.28	951	13.71	7.69		
4	2.0	1	11	D	4	79	269	98	265	9.28	951	13.71	7.69		
4	2.0	1	11	D	4	79	241	97	261	9.28	951	13.71	7.69		
4	3.0	1	11	D	4	68	231	99	261	9.28	951	10.10	7.69		
4	3.0	1	11	D	4	74	249	98	260	9.28	951	10.10	7.69		
4	3.0	1	11	D	4	71	237	102	269	9.28	951	10.10	7.69		
4	1.0	2	11	D	4	79	235	92	239	6.78	930	8.89	7.74		
4	1.0	2	11	D	4	79	230	96	253	6.78	930	8.89	7.74		
4	1.0	2	11	D	4	85	222	93	257	6.78	930	8.89	7.74		
4	1.0	2	11	D	4	80	219	90	243	6.74	931	8.83	7.74		
4	1.0	2	11	D	4	82	228	92	239	6.74	931	8.83	7.74		
4	1.0	2	11	D	4	80	226	88	240	6.74	931	8.83	7.74		
4	2.0	2	11	D	4	81	223	91	239	7.41	934	6.23	7.86		
4	2.0	2	11	D	4	75	228	88	256	7.41	934	6.23	7.86		
4	2.0	2	11	D	4	80	225	92	254	7.41	934	6.23	7.86		
4	3.0	2	11	D	4										
4	3.0	2	11	D	4										
4	3.0	2	11	D	4										
4	1.0	3	11	D	4	80	220	88	228	6.82	918	9.19	7.73		
4	1.0	3	11	D	4	81	220	90	238	6.82	918	9.19	7.73		
4	1.0	3	11	D	4	78	230	100	240	6.82	918	9.19	7.73		
4	1.0	3	11	D	4	78	221	85	233	7.04	924	8.39	7.73		
4	1.0	3	11	D	4	78	226	92	228	7.04	924	8.39	7.73		
4	1.0	3	11	D	4	79	224	92	232	7.04	924	8.39	7.73		
4	2.0	3	11	D	4	72	223	91	231	7.62	928	10.07	7.88		

4	2.0	3	11	D	4	74	208	87	243	7.62	928	10.07	7.88		
4	2.0	3	11	D	4	74	220	89	236	7.62	928	10.07	7.88		
4	3.0	3	11	D	4										
4	3.0	3	11	D	4										
4	3.0	3	11	D	4										
4	1.0	4	11	D	4	84	229	87	238	6.17	922	9.27	7.8		
4	1.0	4	11	D	4	85	213	93	237	6.17	922	9.27	7.8		
4	1.0	4	11	D	4	84	222	91	250	6.17	922	9.27	7.8		
4	1.0	4	11	D	4	80	221	94	250	6.16	925	9.86	7.76		
4	1.0	4	11	D	4	88	225	90	253	6.16	925	9.86	7.76		
4	1.0	4	11	D	4	90	238	94	238	6.16	925	9.86	7.76		
4	2.0	4	11	D	4	84	217	88	240	6.72	931	10.02	7.95		
4	2.0	4	11	D	4	80	230	92	236	6.72	931	10.02	7.95		
4	2.0	4	11	D	4	83	225	94	260	6.72	931	10.02	7.95		
4	3.0	4	11	D	4										
4	3.0	4	11	D	4										
4	3.0	4	11	D	4										
4	1.0	5	11	D	4	79	219	93	247	6.51	921		7.76	25	17
4	1.0	5	11	D	4	81	236	91	238	6.51	921		7.76	25	
4	1.0	5	11	D	4	84	236	97	248	6.51	921		7.76	25	17
4	1.0	5	11	D	4	74	226	91	246	6.57	916		7.63	25	17
4	1.0	5	11	D	4	82	227	96	245	6.57	916		7.63	25	17
4	1.0	5	11	D	4	81	228	89	241	6.57	916		7.63	25	17
4	2.0	5	11	D	4	80	231	95	255	7.12	917		7.89		
4	2.0	5	11	D	4	78	241	93	247	7.12	917		7.89		
4	2.0	5	11	D	4	81	224	97	254	7.12	917		7.89		
4	3.0	5	11	D	4	77	216	89	251	6.27	921		8.58		
4	3.0	5	11	D	4	76	229	93	245	6.27	921		8.58		
4	3.0	5	11	D	4	78	221	92	257	6.27	921		8.58		
4	1.0	12	11	D	4	72	220	88	229	6.7	922	9.38	7.6	125	210
4	1.0	12	11	D	4	71	207	89	252	6.7	922	9.38	7.6	125	210
4	1.0	12	11	D	4	69	202	91	240	6.7	922	9.38	7.6	125	210
4	1.0	12	11	D	4	73	213	87	258	6.6	911	9.61	7.57	125	210
4	1.0	12	11	D	4	78	211	89	247	6.6	911	9.61	7.57	125	210
4	1.0	12	11	D	4	75	207	92	238	6.6	911	9.61	7.57	125	210
4	2.0	12	11	D	4	78	216	92	244	7.71	924	9.97	7.8	189	244
4	2.0	12	11	D	4	75	233	94	249	7.71	924	9.97	7.8	189	244
4	2.0	12	11	D	4	77	226	96	241	7.71	924	9.97	7.8	189	244
4	3.0	12	11	D	4	75	210	93	238	7.84	915	6.76	8.47	9	
4	3.0	12	11	D	4	80	208	93	253	7.84	915	6.76	8.47	9	
4	3.0	12	11	D	4	80	210	91	243	7.84	915	6.76	8.47	9	
4	1.0	19	11	D	4	70	205	92	243	6.92	917	5.96	7.4	135	254
4	1.0	19	11	D	4	75	198	87	228	6.92	917	5.96	7.4	135	254
4	1.0	19	11	D	4	67	204	90	235	6.92	917	5.96	7.4	135	254
4	1.0	19	11	D	4	71	200	91	235	7.11	908	5.40	7.38	135	254
4	1.0	19	11	D	4	73	204	90	249	7.11	908	5.40	7.38	135	254
4	1.0	19	11	D	4	74	204	91	232	7.11	908	5.40	7.38	135	254
4	2.0	19	11	D	4	77	201	88	234	7.54	914	9.10	7.63	189	285
4	2.0	19	11	D	4	76	211	90	229	7.54	914	9.10	7.63	189	285
4	2.0	19	11	D	4	78	196	92	244	7.54	914	9.10	7.63	189	285
4	3.0	19	11	D	4	68	215	91	251	8.54	920	7.86	8.39	12	
4	3.0	19	11	D	4	79	216	92	254	8.54	920	7.86	8.39	12	
4	3.0	19	11	D	4	81	224	92	245	8.54	920	7.86	8.39	12	
4	1.0	26	11	D	4	78	193	87	219	7.28	912	7.99	7.47	135	254
4	1.0	26	11	D	4	72	188	87	238	7.28	912	7.99	7.47	135	254
4	1.0	26	11	D	4	84	193	92	230	7.28	912	7.99	7.47	135	254
4	1.0	26	11	D	4	79	196	92	223	7.11	924	5.95	7.45	135	254
4	1.0	26	11	D	4	75	203	92	237	7.11	924	5.95	7.45	135	254
4	1.0	26	11	D	4	72	193	94	234	7.11	924	5.95	7.45	135	254
4	2.0	26	11	D	4	73	195	86	226	7.65	907	7.98	7.67	189	285
4	2.0	26	11	D	4	72	207	91	239	7.65	907	7.98	7.67	189	285
4	2.0	26	11	D	4	74	198	93	232	7.65	907	7.98	7.67	189	285
4	3.0	26	11	D	4	79	211	87	226	8.18	914	7.85	8.3	12	1
4	3.0	26	11	D	4	78	206	92	232	8.18	914	7.85	8.3	12	1
4	3.0	26	11	D	4	80	206	92	247	8.18	914	7.85	8.3	12	1

5	1.0	1	11	D	2	84	351	164	431	8.47	537	3.89	7.83		
5	1.0	1	11	D	2	100	318	187	462	8.47	537	3.89	7.83		
5	1.0	1	11	D	2	82	323	167	457	8.47	537	3.89	7.83		
5	2.0	1	11	D	2	90	236	164	424	8.47	537	5.16	7.83		
5	2.0	1	11	D	2	102	247	166	426	8.47	537	5.16	7.83		
5	2.0	1	11	D	2	85	297	164	456	8.47	537	5.16	7.83		
5	3.0	1	11	D	2	74	249	164	424	8.47	537	4.56	7.83		
5	3.0	1	11	D	2	72	316	164	426	8.47	537	4.56	7.83		
5	3.0	1	11	D	2	102	244	164	531	8.47	537	4.56	7.83		
5	1.0	2	11	D	2	96	319	158	405	8.23	5.25	2.06	7.89		
5	1.0	2	11	D	2	91	332	153	435	8.23	5.25	2.06	7.89		
5	1.0	2	11	D	2	80	326	155	429	8.23	5.25	2.06	7.89		
5	2.0	2	11	D	2	95	299	156	420	8.11	530	3.66	7.85		
5	2.0	2	11	D	2	90	301	168	419	8.11	530	3.66	7.85		
5	2.0	2	11	D	2	83	316	159	404	8.11	530	3.66	7.85		
5	2.0	2	11	D	2	85	322	155	425	8.15	532	5.07	7.9		
5	2.0	2	11	D	2	85	321	156	434	8.15	532	5.07	7.9		
5	2.0	2	11	D	2	100	314	158	434	8.15	532	5.07	7.9		
5	3.0	2	11	D	2										
5	3.0	2	11	D	2										
5	3.0	2	11	D	2										
5	1.0	3	11	D	2	87	292	170	424	7.92	529	4.73	7.8		
5	1.0	3	11	D	2	89	282	158	412	7.92	529	4.73	7.8		
5	1.0	3	11	D	2	89	297	158	408	7.92	529	4.73	7.8		
5	2.0	3	11	D	2	87	322	155	411	8.09	528	4.94	7.78		
5	2.0	3	11	D	2	87	342	157	417	8.09	528	4.94	7.78		
5	2.0	3	11	D	2	97	320	153	412	8.09	528	4.94	7.78		
5	2.0	3	11	D	2	84	333	152	411	7.94	527	4.93	7.83		
5	2.0	3	11	D	2	89	350	155	432	7.94	527	4.93	7.83		
5	2.0	3	11	D	2	106	322	155	412	7.94	527	4.93	7.83		
5	3.0	3	11	D	2										
5	3.0	3	11	D	2										
5	3.0	3	11	D	2										
5	1.0	4	11	D	2	84	316	155	409	6.9	531	3.45	7.67	260	61
5	1.0	4	11	D	2	86	333	155	422	6.9	531	3.45	7.67	260	61
5	1.0	4	11	D	2	76	305	161	437	6.9	531	3.45	7.67	260	61
5	2.0	4	11	D	2	99	358	155	405	6.98	529	3.26	7.73		
5	2.0	4	11	D	2	119	356	155	417	6.98	529	3.26	7.73		
5	2.0	4	11	D	2	101	356	161	419	6.98	529	3.26	7.73		
5	2.0	4	11	D	2	102	336	155	409	7.32	530	3.24	7.69		
5	2.0	4	11	D	2	101	344	163	433	7.32	530	3.24	7.69		
5	2.0	4	11	D	2	99	336	157	414	7.32	530	3.24	7.69		
5	3.0	4	11	D	2										
5	3.0	4	11	D	2										
5	3.0	4	11	D	2										
5	1.0	5	11	D	2	74	326	150	429	6.73	522	3.07	7.62	300	169
5	1.0	5	11	D	2	74	268	157	426	6.73	522	3.07	7.62	300	169
5	1.0	5	11	D	2	89	283	155	421	6.73	522	3.07	7.62	300	169
5	2.0	5	11	D	2	91	326	155	429	7.44	524	3.07	7.67	5	
5	2.0	5	11	D	2	104	377	155	426	7.44	524	3.07	7.67	5	
5	2.0	5	11	D	2	96	335	168	433	7.44	524	3.07	7.67	5	
5	2.0	5	11	D	2	84	337	157	412	7.64	530	3.39	7.67	5	
5	2.0	5	11	D	2	103	337	160	433	7.64	530	3.39	7.67	5	
5	2.0	5	11	D	2	101	333	160	453	7.64	530	3.39	7.67	5	
5	3.0	5	11	D	2	99	320	153	407	7.07	528	2.90	8	2	2
5	3.0	5	11	D	2	96	352	163	421	7.07	528	2.90	8	2	2
5	3.0	5	11	D	2	94	325	157	429	7.07	528	2.90	8	2	2
5	1.0	12	11	D	2	98	334	157	441	6.49	534	3.09	7.42	>300	169
5	1.0	12	11	D	2	104	267	157	412	6.49	534	3.09	7.42	>300	169
5	1.0	12	11	D	2	104	324	170	434	6.49	534	3.09	7.42	>300	169
5	2.0	12	11	D	2	72	275	150	421	6.7	531	3.42	7.6	32	18
5	2.0	12	11	D	2	73	287	160	441	6.7	531	3.42	7.6	32	18
5	2.0	12	11	D	2	70	311	154	432	6.7	531	3.42	7.6	32	18
5	2.0	12	11	D	2	88	319	154	444	6.7	526	3.40	7.56	32	18
5	2.0	12	11	D	2	82	326	159	456	6.7	526	3.40	7.56	32	18

5	2.0	12	11	D	2	88	306	164	432	6.7	526	3.40	7.56	32	18
5	3.0	12	11	D	2	95	334	160	426	6.79	534	3.57	7.82	4	13
5	3.0	12	11	D	2	90	329	154	437	6.79	534	3.57	7.82	4	13
5	3.0	12	11	D	2	95	317	162	424	6.79	534	3.57	7.82	4	13
5	1.0	19	11	D	2	92	232	157	407	7.85	536	3.46	7.4	>300	178
5	1.0	19	11	D	2	87	260	157	420	7.85	536	3.46	7.4	>300	178
5	1.0	19	11	D	2	91	262	161	430	7.85	536	3.46	7.4	>300	178
5	2.0	19	11	D	2	104	302	155	427	8.2	535	3.93	7.53	39	20
5	2.0	19	11	D	2	104	290	157	453	8.2	535	3.93	7.53	39	20
5	2.0	19	11	D	2	92	308	161	453	8.2	535	3.93	7.53	39	20
5	2.0	19	11	D	2	87	273	155	460	8.25	530	3.52	7.53	39	20
5	2.0	19	11	D	2	91	247	157	412	8.25	530	3.52	7.53	39	20
5	2.0	19	11	D	2	91	267	153	435	8.25	530	3.52	7.53	39	20
5	3.0	19	11	D	2	105	308	166	430	8.36	530	4.76	7.75	6	15
5	3.0	19	11	D	2	104	318	153	399	8.36	530	4.76	7.75	6	15
5	3.0	19	11	D	2	109	310	161	443	8.36	530	4.76	7.75	6	15
5	1.0	26	11	D	2	66	233	159	413	8.56	539	3.11	7.34	>300	178
5	1.0	26	11	D	2	102	256	156	455	8.56	539	3.11	7.34	>300	178
5	1.0	26	11	D	2	65	237	160	431	8.56	539	3.11	7.34	>300	178
5	2.0	26	11	D	2	68	256	147	400	8.39	545	3.33	7.4	39	22
5	2.0	26	11	D	2	71	259	156	415	8.39	545	3.33	7.4	39	22
5	2.0	26	11	D	2	70	264	159	415	8.39	545	3.33	7.4	39	22
5	2.0	26	11	D	2	79	250	162	412	8.59	540	3.23	7.4	39	22
5	2.0	26	11	D	2	73	258	151	423	8.59	540	3.23	7.4	39	22
5	2.0	26	11	D	2	68	254	162	407	8.59	540	3.23	7.4	39	22
5	3.0	26	11	D	2	86	288	159	402	8.76	540	3.90	7.77	6	15
5	3.0	26	11	D	2	92	324	156	421	8.76	540	3.90	7.77	6	15
5	3.0	26	11	D	2	86	301	156	425	8.76	540	3.90	7.77	6	15
6	3.0	1	11	D	1	3	31	3	8			0.33			
6	3.0	1	11	D	1	3	31	3	8			0.33			
6	3.0	1	11	D	1	3	31	3	8			0.33			
6	3.0	1	11	D	1	3	31	3	8			0.33			
6	1.0	1	11	D	1	2	22	2	7			0.18			
6	1.0	1	11	D	1	2	22	2	7			0.18			
6	1.0	1	11	D	1	2	22	2	7			0.18			
6	1.0	1	11	D	1	2	22	2	7			0.18			
6	2.0	1	11	D	1	1	23	3	5			0.34			
6	2.0	1	11	D	1	1	23	3	5			0.34			
6	2.0	1	11	D	1	1	23	3	5			0.34			
6	2.0	1	11	D	1	1	23	3	5			0.34			
6	3.0	2	11	D	1	2	32	4	36						
6	1.0	2	11	D	1	2	37	3	35						
6	2.0	2	11	D	1	3	48	3	41						
6	3.0	4	11	D	1	3	28	3	45			0.51			
6	1.0	4	11	D	1	3	26	6	74			0.33			
6	2.0	4	11	D	1	2	31	5	40			0.47			
6	3.0	5	11	D	1	3	25	4	50			0.36			
6	1.0	5	11	D	1	3	37	4	65			0.36			
6	2.0	5	11	D	1	4	33	4	43			0.41			
6	3.0	12	11	D	1	3	35	4	56			0.56			
6	1.0	12	11	D	1	3	36	5	71			0.55			
6	2.0	12	11	D	1	6	41	5	76			0.47			
6	3.0	19	11	D	1	3	25	5	101			0.57		1	
6	1.0	19	11	D	1	3	20	5	97			0.59			
6	2.0	19	11	D	1	3	17	5	66			0.68			1
6	3.0	26	11	D	1	4	22	4	93			0.93		1	
6	1.0	26	11	D	1	3	31	5	114			0.92			
6	2.0	26	11	D	1	4	26	5	83			0.84			1

APPENDIX 4

							Fluorescence Intensity (AFU)				mgl-1				CFU	
Name	Date	Fraction	Day	Temp	Light	Dilution	T1Int	T2Int	Cint	Aint	DO	Cond	TOC	pH	10-1 YEA	10-1 R2A
1	08/05/06	3.0	1	4	D	0	36	85	88	190	10.4	650		8.06		
1	08/05/06	3.0	1	4	D	0	36	115	81	187	10.4	650		8.06		
1	08/05/06	3.0	1	4	D	0	31	85	81	201	10.4	650		8.06		
1	08/05/06	1.0	1	4	D	0	40	112	93	206	10.4	650	2.97	8.06		
1	08/05/06	1.0	1	4	D	0	42	132	90	202	10.4	650	2.97	8.06		
1	08/05/06	1.0	1	4	D	0	39	118	94	217	10.4	650	2.97	8.06		
1	08/05/06	2.0	1	4	D	0	37	109	91	195	10.4	650	1.63	8.06		
1	08/05/06	2.0	1	4	D	0	42	106	93	199	10.4	650	1.63	8.06		
1	08/05/06	2.0	1	4	D	0	37	95	91	209	10.4	650	1.63	8.06		
1	08/05/06	3.0	1	20	D	0	36	85	88	190	10.4	650		8.06		
1	08/05/06	3.0	1	20	D	0	36	115	81	187	10.4	650		8.06		
1	08/05/06	3.0	1	20	D	0	31	85	81	201	10.4	650		8.06		
1	08/05/06	1.0	1	20	D	0	40	112	93	206	10.4	650	2.97	8.06		
1	08/05/06	1.0	1	20	D	0	42	132	90	202	10.4	650	2.97	8.06		
1	08/05/06	1.0	1	20	D	0	39	118	94	217	10.4	650	2.97	8.06		
1	08/05/06	2.0	1	20	D	0	37	109	91	195	10.4	650	1.63	8.06		
1	08/05/06	2.0	1	20	D	0	42	106	93	199	10.4	650	1.63	8.06		
1	08/05/06	2.0	1	20	D	0	37	95	91	209	10.4	650	1.63	8.06		
1	09/05/06	3.0	2	4	D	0	37	95	85	187	7.35	624		8.23		
1	09/05/06	3.0	2	4	D	0	36	115	87	190	7.35	624		8.23		
1	09/05/06	3.0	2	4	D	0	35	112	93	205	7.35	624		8.23		
1	09/05/06	1.0	2	4	D	0	43	107	95	217	8.85	645	4.55	8.18		
1	09/05/06	1.0	2	4	D	0	39	102	91	212	8.85	645	4.55	8.18		
1	09/05/06	1.0	2	4	D	0	39	102	93	213	8.85	645	4.55	8.18		
1	09/05/06	1.0	2	4	D	0	41	98	88	206	9.05	643	4.67	8.08		
1	09/05/06	1.0	2	4	D	0	38	107	89	197	9.05	643	4.67	8.08		
1	09/05/06	1.0	2	4	D	0	46	105	91	202	9.05	643	4.67	8.08		
1	09/05/06	2.0	2	4	D	0	37	101	88	197	8.52	634	3.77	8.16		
1	09/05/06	2.0	2	4	D	0	39	110	91	208	8.52	634	3.77	8.16		
1	09/05/06	2.0	2	4	D	0	32	99	90	199	8.52	634	3.77	8.16		
1	09/05/06	3.0	2	20	D	0	43	111	95	239	6.63	635	3.93	8.25		
1	09/05/06	3.0	2	20	D	0	40	103	93	235	6.63	635	3.93	8.25		
1	09/05/06	3.0	2	20	D	0	35	94	94	244	6.63	635	3.93	8.25		
1	09/05/06	1.0	2	20	D	0	44	113	95	224	7.86	645	4.15	8.12		
1	09/05/06	1.0	2	20	D	0	46	100	96	236	7.86	645	4.15	8.12		
1	09/05/06	1.0	2	20	D	0	44	98	97	233	7.86	645	4.15	8.12		
1	09/05/06	1.0	2	20	D	0	48	85	95	225	7.85	644	4.08	8.15		
1	09/05/06	1.0	2	20	D	0	41	100	100	222	7.85	644	4.08	8.15		
1	09/05/06	1.0	2	20	D	0	41	112	98	225	7.85	644	4.08	8.15		
1	09/05/06	2.0	2	20	D	0	43	101	100	241	8.09	638	4.09	8.17	2	
1	09/05/06	2.0	2	20	D	0	43	118	99	234	8.09	638	4.09	8.17	2	
1	09/05/06	2.0	2	20	D	0	43	109	95	241	8.09	638	4.09	8.17	2	
1	10/05/06	3.0	3	4	D	0										
1	10/05/06	3.0	3	4	D	0										
1	10/05/06	3.0	3	4	D	0										
1	10/05/06	1.0	3	4	D	0	53	120	104	222	8.72	644	4.11	8.17		
1	10/05/06	1.0	3	4	D	0	45	129	98	228	8.72	644	4.11	8.17		
1	10/05/06	1.0	3	4	D	0	46	126	98	211	8.72	644	4.11	8.17		
1	10/05/06	1.0	3	4	D	0	54	132	97	224	8.63	644	4.46	8.17		
1	10/05/06	1.0	3	4	D	0	63	141	95	236	8.63	644	4.46	8.17		
1	10/05/06	1.0	3	4	D	0	68	146	100	217	8.63	644	4.46	8.17		
1	10/05/06	2.0	3	4	D	0	39	116	93	214	8.36	635	3.59	8.17		
1	10/05/06	2.0	3	4	D	0	39	115	94	212	8.36	635	3.59	8.17		
1	10/05/06	2.0	3	4	D	0	38	122	98	228	8.36	635	3.59	8.17		
1	10/05/06	3.0	3	20	D	0										
1	10/05/06	3.0	3	20	D	0										
1	10/05/06	3.0	3	20	D	0										
1	10/05/06	1.0	3	20	D	0	40	110	103	246	7.4	645	3.90	8.08	14	5
1	10/05/06	1.0	3	20	D	0	44	106	103	268	7.4	645	3.90	8.08	14	5
1	10/05/06	1.0	3	20	D	0	42	108	100	235	7.4	645	3.90	8.08	14	5
1	10/05/06	1.0	3	20	D	0	36	105	102	217	7.11	646	4.19	8.07	14	5
1	10/05/06	1.0	3	20	D	0	40	104	102	224	7.11	646	4.19	8.07	14	5
1	10/05/06	1.0	3	20	D	0	40	104	103	221	7.11	646	4.19	8.07	14	5
1	10/05/06	2.0	3	20	D	0	39	102	102	261	7.6	633	4.25	8.08	8	1
1	10/05/06	2.0	3	20	D	0	46	104	105	236	7.6	633	4.25	8.08	8	1
1	10/05/06	2.0	3	20	D	0	40	113	103	241	7.6	633	4.25	8.08	8	1
1	11/05/06	3.0	4	4	D	0										
1	11/05/06	3.0	4	4	D	0										

1	11/05/06	3.0	4	4	D	0										
1	11/05/06	1.0	4	4	D	0	54	122	97	221	8.2	645	1.28	8.09		
1	11/05/06	1.0	4	4	D	0	52	121	97	211	8.2	645	1.28	8.09		
1	11/05/06	1.0	4	4	D	0	49	118	96	213	8.2	645	1.28	8.09		
1	11/05/06	1.0	4	4	D	0	51	118	93	213	8.17	645	2.61	8.07		
1	11/05/06	1.0	4	4	D	0	48	113	96	220	8.17	645	2.61	8.07		
1	11/05/06	1.0	4	4	D	0	52	114	100	209	8.17	645	2.61	8.07		
1	11/05/06	2.0	4	4	D	0	55	127	96	219	8.1	630	0.04	8.11		
1	11/05/06	2.0	4	4	D	0	44	120	99	222	8.1	630	0.04	8.11		
1	11/05/06	2.0	4	4	D	0	51	132	99	210	8.1	630	0.04	8.11		
1	11/05/06	3.0	4	20	D	0										
1	11/05/06	3.0	4	20	D	0										
1	11/05/06	3.0	4	20	D	0										
1	11/05/06	1.0	4	20	D	0	46	122	103	262	6.44	648	1.99	8.18	14	5
1	11/05/06	1.0	4	20	D	0	55	114	103	265	6.44	648	1.99	8.18	14	5
1	11/05/06	1.0	4	20	D	0	56	131	105	253	6.44	648	1.99	8.18	14	5
1	11/05/06	1.0	4	20	D	0	52	124	108	280	6.83	643	0.82	8.16	14	5
1	11/05/06	1.0	4	20	D	0	49	116	117	264	6.83	643	0.82	8.16	14	5
1	11/05/06	1.0	4	20	D	0	52	117	112	277	6.83	643	0.82	8.16	14	5
1	11/05/06	2.0	4	20	D	0	45	115	106	278	7.44	636	0.81	8.29	8	1
1	11/05/06	2.0	4	20	D	0	49	109	105	268	7.44	636	0.81	8.29	8	1
1	11/05/06	2.0	4	20	D	0	48	106	106	280	7.44	636	0.81	8.29	8	1
1	12/05/06	3.0	5	4	D	0										
1	12/05/06	3.0	5	4	D	0										
1	12/05/06	3.0	5	4	D	0										
1	12/05/06	1.0	5	4	D	0	45	116	94	219	8.36	645		8.22		
1	12/05/06	1.0	5	4	D	0	49	135	103	221	8.36	645		8.22		
1	12/05/06	1.0	5	4	D	0	45	129	97	217	8.36	645		8.22		
1	12/05/06	1.0	5	4	D	0	52	113	99	236	8.24	645		8.2		
1	12/05/06	1.0	5	4	D	0	54	126	101	242	8.24	645		8.2		
1	12/05/06	1.0	5	4	D	0	48	117	99	229	8.24	645		8.2		
1	12/05/06	2.0	5	4	D	0	51	135	92	212	8.01	632		8.26		
1	12/05/06	2.0	5	4	D	0	48	122	96	215	8.01	632		8.26		
1	12/05/06	2.0	5	4	D	0	50	115	93	223	8.01	632		8.26		
1	12/05/06	3.0	5	20	D	0									2	1
1	12/05/06	3.0	5	20	D	0									2	1
1	12/05/06	3.0	5	20	D	0									2	1
1	12/05/06	1.0	5	20	D	0	52	118	102	240	6.75	646		7.98	51	55
1	12/05/06	1.0	5	20	D	0	41	106	105	259	6.75	646		7.98	51	55
1	12/05/06	1.0	5	20	D	0	59	125	104	251	6.75	646		7.98	51	55
1	12/05/06	1.0	5	20	D	0	47	113	111	266	5.8	645		8	51	55
1	12/05/06	1.0	5	20	D	0	51	109	111	262	5.8	645		8	51	55
1	12/05/06	1.0	5	20	D	0	48	127	108	259	5.8	645		8	51	55
1	12/05/06	2.0	5	20	D	0	48	109	108	267	7.07	637		7.94	46	28
1	12/05/06	2.0	5	20	D	0	54	123	107	269	7.07	637		7.94	46	28
1	12/05/06	2.0	5	20	D	0	43	150	108	301	7.07	637		7.94	46	28
1	19/05/06	3.0	12	4	D	0	47	106	88	223	8.81	612	6.51	8.35		
1	19/05/06	3.0	12	4	D	0	46	124	89	210	8.81	612	6.51	8.35		
1	19/05/06	3.0	12	4	D	0	46	110	87	205	8.81	612	6.51	8.35		
1	19/05/06	1.0	12	4	D	0	55	114	100	240	8.94	649	5.95	8.18	8	10
1	19/05/06	1.0	12	4	D	0	56	118	103	227	8.94	649	5.95	8.18	8	10
1	19/05/06	1.0	12	4	D	0	50	134	99	231	8.94	649	5.95	8.18	8	10
1	19/05/06	1.0	12	4	D	0	52	123	101	232	8.72	649	4.82	8.15	8	10
1	19/05/06	1.0	12	4	D	0	43	107	103	236	8.72	649	4.82	8.15	8	10
1	19/05/06	1.0	12	4	D	0	48	115	101	236	8.72	649	4.82	8.15	8	10
1	19/05/06	2.0	12	4	D	0	52	115	107	232	8.82	639	5.90	8.25		1
1	19/05/06	2.0	12	4	D	0	48	106	104	238	8.82	639	5.90	8.25		1
1	19/05/06	2.0	12	4	D	0	50	115	102	232	8.82	639	5.90	8.25		1
1	19/05/06	3.0	12	20	D	0	57	139	117	271	8.13	640	6.52	7.85	4	4
1	19/05/06	3.0	12	20	D	0	59	129	116	292	8.13	640	6.52	7.85	4	4
1	19/05/06	3.0	12	20	D	0	51	142	112	284	8.13	640	6.52	7.85	4	4
1	19/05/06	1.0	12	20	D	0	49	127	113	282	5.06	653	6.46	7.54	86	255
1	19/05/06	1.0	12	20	D	0	44	136	109	270	5.06	653	6.46	7.54	86	255
1	19/05/06	1.0	12	20	D	0	48	116	116	280	5.06	653	6.46	7.54	86	255
1	19/05/06	1.0	12	20	D	0	46	126	115	272	6.87	652	5.06	7.6	86	255
1	19/05/06	1.0	12	20	D	0	46	119	114	271	6.87	652	5.06	7.6	86	255
1	19/05/06	1.0	12	20	D	0	51	123	118	284	6.87	652	5.06	7.6	86	255
1	19/05/06	2.0	12	20	D	0	52	128	109	268	7.7	639	6.23	7.87	80	28
1	19/05/06	2.0	12	20	D	0	57	136	112	266	7.7	639	6.23	7.87	80	28
1	19/05/06	2.0	12	20	D	0	54	138	108	265	7.7	639	6.23	7.87	80	28
1	26/05/06	3.0	19	4	D	0	46	107	94	200	8.3	605	5.11	8.56		
1	26/05/06	3.0	19	4	D	0	45	130	88	203	8.3	605	5.11	8.56		
1	26/05/06	3.0	19	4	D	0	45	136	85	219	8.3	605	5.11	8.56		
1	26/05/06	1.0	19	4	D	0	46	105	100	221	8.52	637	3.75	8	19	26
1	26/05/06	1.0	19	4	D	0	51	113	104	222	8.52	637	3.75	8	19	26

1	26/05/06	1.0	19	4	D	0	42	111	105	222	8.52	637	3.75	8	19	26
1	26/05/06	1.0	19	4	D	0	50	110	104	236	8.66	635	5.82	7.86	19	26
1	26/05/06	1.0	19	4	D	0	45	113	99	230	8.66	635	5.82	7.86	19	26
1	26/05/06	1.0	19	4	D	0	51	118	98	249	8.66	635	5.82	7.86	19	26
1	26/05/06	2.0	19	4	D	0	45	131	94	222	9.36	628	6.02	8.11	5	2
1	26/05/06	2.0	19	4	D	0	46	115	95	216	9.36	628	6.02	8.11	5	2
1	26/05/06	2.0	19	4	D	0	51	131	99	225	9.36	628	6.02	8.11	5	2
1	26/05/06	3.0	19	20	D	0	58	140	106	272	6.85	657	8.75	7.16	6	5
1	26/05/06	3.0	19	20	D	0	56	144	106	259	6.85	657	8.75	7.16	6	5
1	26/05/06	3.0	19	20	D	0	52	126	108	241	6.85	657	8.75	7.16	6	5
1	26/05/06	1.0	19	20	D	0	46	101	112	265	6.72	648	7.78	7.34	105	300
1	26/05/06	1.0	19	20	D	0	49	127	116	268	6.72	648	7.78	7.34	105	300
1	26/05/06	1.0	19	20	D	0	41	115	115	282	6.72	648	7.78	7.34	105	300
1	26/05/06	1.0	19	20	D	0	49	108	121	321	7.53	646	6.18	7.48	105	300
1	26/05/06	1.0	19	20	D	0	56	94	120	339	7.53	646	6.18	7.48	105	300
1	26/05/06	1.0	19	20	D	0	56	99	126	323	7.53	646	6.18	7.48	105	300
1	26/05/06	2.0	19	20	D	0	44	146	120	338	7.9	641	5.34	7.67	97	179
1	26/05/06	2.0	19	20	D	0	53	120	121	331	7.9	641	5.34	7.67	97	179
1	26/05/06	2.0	19	20	D	0	48	127	121	341	7.9	641	5.34	7.67	97	179
1	02/06/06	3.0	26	4	D	0	49	117	95	224	8.88	610	5.61	8.6		1
1	02/06/06	3.0	26	4	D	0	44	118	97	228	8.88	610	5.61	8.6		1
1	02/06/06	3.0	26	4	D	0	43	119	91	238	8.88	610	5.61	8.6		1
1	02/06/06	1.0	26	4	D	0	49	98	95	226	8.88	639	7.51	8.01	42	59
1	02/06/06	1.0	26	4	D	0	51	106	106	222	8.88	639	7.51	8.01	42	59
1	02/06/06	1.0	26	4	D	0	51	104	113	231	8.88	639	7.51	8.01	42	59
1	02/06/06	1.0	26	4	D	0	46	130	107	252	8.46	632	9.08	7.97	42	59
1	02/06/06	1.0	26	4	D	0	52	108	112	256	8.46	632	9.08	7.97	42	59
1	02/06/06	1.0	26	4	D	0	58	112	104	249	8.46	632	9.08	7.97	42	59
1	02/06/06	2.0	26	4	D	0	38	90	95	227	9.18	629	7.66	8.23	16	10
1	02/06/06	2.0	26	4	D	0	44	102	101	228	9.18	629	7.66	8.23	16	10
1	02/06/06	2.0	26	4	D	0	54	100	101	218	9.18	629	7.66	8.23	16	10
1	02/06/06	3.0	26	20	D	0	57	153	116	309	7.13	620	11.62	7.4	11	5
1	02/06/06	3.0	26	20	D	0	61	153	123	309	7.13	620	11.62	7.4	11	5
1	02/06/06	3.0	26	20	D	0	65	142	120	308	7.13	620	11.62	7.4	11	5
1	02/06/06	1.0	26	20	D	0	44	113	118	296	6.72	643	10.32	7.24	127	300
1	02/06/06	1.0	26	20	D	0	47	117	117	285	6.72	643	10.32	7.24	127	300
1	02/06/06	1.0	26	20	D	0	52	112	130	289	6.72	643	10.32	7.24	127	300
1	02/06/06	1.0	26	20	D	0	55	104	120	289	6.64	644	7.47	7.23	127	300
1	02/06/06	1.0	26	20	D	0	49	119	121	303	6.64	644	7.47	7.23	127	300
1	02/06/06	1.0	26	20	D	0	49	101	124	298	6.64	644	7.47	7.23	127	300
1	02/06/06	2.0	26	20	D	0	65	144	124	280	6.67	634	7.21	7.27	114	300
1	02/06/06	2.0	26	20	D	0	52	139	114	284	6.67	634	7.21	7.27	114	300
1	02/06/06	2.0	26	20	D	0	55	137	116	279	6.67	634	7.21	7.27	114	300
2	05/06/06	1.0	1	20	D	0	44	130	78	172	10.58	734	4.86	7.82		
2	05/06/06	1.0	1	20	D	0	47	118	81	181	10.58	734	4.86	7.82		
2	05/06/06	1.0	1	20	D	0	43	119	80	178	10.58	734	4.86	7.82		
2	05/06/06	2.0	1	20	D	0	42	124	81	174	10.58	738		7.9		
2	05/06/06	2.0	1	20	D	0	56	133	86	175	10.58	738		7.9		
2	05/06/06	2.0	1	20	D	0	55	128	80	184	10.58	738		7.9		
2	05/06/06	3.0	1	20	D	0	37	125	75	160	10.58	724		8.07		
2	05/06/06	3.0	1	20	D	0	48	125	75	182	10.58	724		8.07		
2	05/06/06	3.0	1	20	D	0	48	133	75	170	10.58	724		8.07		
2	05/06/06	1.0	1	4	D	0	44	130	78	172	10.58	734	4.86	7.82		
2	05/06/06	1.0	1	4	D	0	47	118	81	181	10.58	734	4.86	7.82		
2	05/06/06	1.0	1	4	D	0	43	119	80	178	10.58	734	4.86	7.82		
2	05/06/06	2.0	1	4	D	0	42	124	81	174	10.58	738		7.9		
2	05/06/06	2.0	1	4	D	0	56	133	86	175	10.58	738		7.9		
2	05/06/06	2.0	1	4	D	0	55	128	80	184	10.58	738		7.9		
2	05/06/06	3.0	1	4	D	0	37	125	75	160	10.58	724		8.07		
2	05/06/06	3.0	1	4	D	0	48	125	75	182	10.58	724		8.07		
2	05/06/06	3.0	1	4	D	0	48	133	75	170	10.58	724		8.07		
2	05/06/06	1.0	1	11	D	0	44	130	78	172	10.58	734	4.86	7.82		
2	05/06/06	1.0	1	11	D	0	47	118	81	181	10.58	734	4.86	7.82		
2	05/06/06	1.0	1	11	D	0	43	119	80	178	10.58	734	4.86	7.82		
2	05/06/06	2.0	1	11	D	0	42	124	81	174	10.58	738		7.9		
2	05/06/06	2.0	1	11	D	0	56	133	86	175	10.58	738		7.9		
2	05/06/06	2.0	1	11	D	0	55	128	80	184	10.58	738		7.9		
2	05/06/06	3.0	1	11	D	0	37	125	75	160	10.58	724		8.07		
2	05/06/06	3.0	1	11	D	0	48	125	75	182	10.58	724		8.07		
2	05/06/06	3.0	1	11	D	0	48	133	75	170	10.58	724		8.07		
2	06/06/06	1.0	2	20	D	0	49	119	82	183	8.03	736	4.66	7.92		
2	06/06/06	1.0	2	20	D	0	45	114	82	184	8.03	736	4.66	7.92		
2	06/06/06	1.0	2	20	D	0	46	141	88	182	8.03	736	4.66	7.92		
2	06/06/06	2.0	2	20	D	0	64	125	82	198	7.98	736	4.42	7.96		
2	06/06/06	2.0	2	20	D	0	51	128	84	192	7.98	736	4.42	7.96		

2	06/06/06	2.0	2	20	D	0	55	129	84	192	7.98	736	4.42	7.96		
2	06/06/06	3.0	2	20	D	0	53	125	87	209	6.98	747	9.56	8.05		
2	06/06/06	3.0	2	20	D	0	56	137	85	211	6.98	747	9.56	8.05		
2	06/06/06	3.0	2	20	D	0	48	128	88	200	6.98	747	9.56	8.05		
2	06/06/06	3.0	2	20	D	0	58	122	86	208	7.06	740	4.37	8.06		
2	06/06/06	3.0	2	20	D	0	47	137	85	210	7.06	740	4.37	8.06		
2	06/06/06	3.0	2	20	D	0	54	131	83	226	7.06	740	4.37	8.06		
2	06/06/06	1.0	2	11	D	0	52	139	82	193	8.18	742	4.70	8		
2	06/06/06	1.0	2	11	D	0	57	141	88	196	8.18	742	4.70	8		
2	06/06/06	1.0	2	11	D	0	51	129	84	198	8.18	742	4.70	8		
2	06/06/06	2.0	2	11	D	0	46	137	84	198	8.11	736	4.67	8.04		
2	06/06/06	2.0	2	11	D	0	46	120	86	200	8.11	736	4.67	8.04		
2	06/06/06	2.0	2	11	D	0	50	149	86	194	8.11	736	4.67	8.04		
2	06/06/06	3.0	2	11	D	0	50	119	79	176	6.8	734	4.23	8.13		
2	06/06/06	3.0	2	11	D	0	50	127	81	180	6.8	734	4.23	8.13		
2	06/06/06	3.0	2	11	D	0	45	117	84	182	6.8	734	4.23	8.13		
2	06/06/06	3.0	2	11	D	0	46	126	80	184	6.83	738	4.39	8.19		
2	06/06/06	3.0	2	11	D	0	49	127	80	191	6.83	738	4.39	8.19		
2	06/06/06	3.0	2	11	D	0	46	123	79	183	6.83	738	4.39	8.19		
2	06/06/06	1.0	2	4	D	0	45	119	79	177	8.22	743	4.65	7.98		
2	06/06/06	1.0	2	4	D	0	48	132	85	180	8.22	743	4.65	7.98		
2	06/06/06	1.0	2	4	D	0	43	140	85	173	8.22	743	4.65	7.98		
2	06/06/06	2.0	2	4	D	0	48	135	84	185	7.89	737	4.67	8.01		
2	06/06/06	2.0	2	4	D	0	50	119	82	186	7.89	737	4.67	8.01		
2	06/06/06	2.0	2	4	D	0	55	133	83	182	7.89	737	4.67	8.01		
2	06/06/06	3.0	2	4	D	0	55	131	82	198	6.81	737	4.11	8.14		
2	06/06/06	3.0	2	4	D	0	52	123	83	197	6.81	737	4.11	8.14		
2	06/06/06	3.0	2	4	D	0	48	128	83	192	6.81	737	4.11	8.14		
2	06/06/06	3.0	2	4	D	0	48	128	81	198	6.56	736	4.25	8.17		
2	06/06/06	3.0	2	4	D	0	49	122	85	188	6.56	736	4.25	8.17		
2	06/06/06	3.0	2	4	D	0	54	140	84	210	6.56	736	4.25	8.17		
2	07/06/06	1.0	3	20	D	0	49	125	81	191	7.43	731		8.23	4	5
2	07/06/06	1.0	3	20	D	0	45	114	84	185	7.43	731		8.23	4	5
2	07/06/06	1.0	3	20	D	0	44	112	83	190	7.43	731		8.23	4	5
2	07/06/06	2.0	3	20	D	0	49	119	82	192	7.42	739		8.01		
2	07/06/06	2.0	3	20	D	0	50	129	85	191	7.42	739		8.01		
2	07/06/06	2.0	3	20	D	0	51	117	83	202	7.42	739		8.01		
2	07/06/06	3.0	3	20	D	0	49	130	86	203	6.82	726		8.04		
2	07/06/06	3.0	3	20	D	0	50	113	84	197	6.82	726		8.04		
2	07/06/06	3.0	3	20	D	0	45	133	84	194	6.82	726		8.04		
2	07/06/06	3.0	3	20	D	0	51	120	79	205	6.62	728		8.06		
2	07/06/06	3.0	3	20	D	0	43	118	85	209	6.62	728		8.06		
2	07/06/06	3.0	3	20	D	0	50	123	80	196	6.62	728		8.06		
2	07/06/06	1.0	3	11	D	0	48	132	83	180	8.11	725		8.03		
2	07/06/06	1.0	3	11	D	0	47	113	85	179	8.11	725		8.03		
2	07/06/06	1.0	3	11	D	0	52	128	84	177	8.11	725		8.03		
2	07/06/06	2.0	3	11	D	0	50	115	78	175	8.12	736		8.06		
2	07/06/06	2.0	3	11	D	0	55	126	85	179	8.12	736		8.06		
2	07/06/06	2.0	3	11	D	0	54	116	81	171	8.12	736		8.06		
2	07/06/06	3.0	3	11	D	0	51	128	84	168	7.42	727		8.22		
2	07/06/06	3.0	3	11	D	0	53	119	77	179	7.42	727		8.22		
2	07/06/06	3.0	3	11	D	0	51	116	82	187	7.42	727		8.22		
2	07/06/06	3.0	3	11	D	0	44	119	77	171	7.28	726		8.22		
2	07/06/06	3.0	3	11	D	0	46	119	75	172	7.28	726		8.22		
2	07/06/06	3.0	3	11	D	0	47	119	78	174	7.28	726		8.22		
2	07/06/06	1.0	3	4	D	0	57	127	82	184	8.47	736		8.04		
2	07/06/06	1.0	3	4	D	0	58	128	81	186	8.47	736		8.04		
2	07/06/06	1.0	3	4	D	0	58	122	85	180	8.47	736		8.04		
2	07/06/06	2.0	3	4	D	0	47	126	82	182	7.85	737		8.1		
2	07/06/06	2.0	3	4	D	0	51	120	82	182	7.85	737		8.1		
2	07/06/06	2.0	3	4	D	0	44	122	79	179	7.85	737		8.1		
2	07/06/06	3.0	3	4	D	0	47	126	79	182	7.03	726		8.22		
2	07/06/06	3.0	3	4	D	0	47	116	79	188	7.03	726		8.22		
2	07/06/06	3.0	3	4	D	0	50	122	78	190	7.03	726		8.22		
2	07/06/06	3.0	3	4	D	0	52	120	77	180	7.11	738		8.26		
2	07/06/06	3.0	3	4	D	0	51	126	79	190	7.11	738		8.26		
2	07/06/06	3.0	3	4	D	0	43	125	80	188	7.11	738		8.26		
2	08/06/06	1.0	4	20	D	0	53	119	85	200	7.09	751	1.69	7.85	37	40
2	08/06/06	1.0	4	20	D	0	51	121	84	195	7.09	751	1.69	7.85	37	40
2	08/06/06	1.0	4	20	D	0	47	118	85	192	7.09	751	1.69	7.85	37	40
2	08/06/06	2.0	4	20	D	0	47	120	85	196	6.83	753	1.93	7.92	38	34
2	08/06/06	2.0	4	20	D	0	44	121	93	201	6.83	753	1.93	7.92	38	34
2	08/06/06	2.0	4	20	D	0	52	121	86	204	6.83	753	1.93	7.92	38	34
2	08/06/06	3.0	4	20	D	0	45	121	85	206	6.22	739	5.28	7.94		
2	08/06/06	3.0	4	20	D	0	44	121	86	199	6.22	739	5.28	7.94		

2	08/06/06	3.0	4	20	D	0	43	123	85	223	6.22	739	5.28	7.94		
2	08/06/06	3.0	4	20	D	0	42	124	80	203	6.7	747	1.86	8.08		
2	08/06/06	3.0	4	20	D	0	48	125	83	199	6.7	747	1.86	8.08		
2	08/06/06	3.0	4	20	D	0	45	128	84	209	6.7	747	1.86	8.08		
2	08/06/06	1.0	4	11	D	0	44	112	81	193	8.6	740	1.76	8.08	6	
2	08/06/06	1.0	4	11	D	0	51	109	83	181	8.6	740	1.76	8.08	6	
2	08/06/06	1.0	4	11	D	0	53	121	85	179	8.6	740	1.76	8.08	6	
2	08/06/06	2.0	4	11	D	0	46	118	77	186	8.52	737	1.50	8.09		
2	08/06/06	2.0	4	11	D	0	49	117	89	183	8.52	737	1.50	8.09		
2	08/06/06	2.0	4	11	D	0	53	120	80	191	8.52	737	1.50	8.09		
2	08/06/06	3.0	4	11	D	0	49	121	75	174	7.73	733	3.35	8.26		
2	08/06/06	3.0	4	11	D	0	45	130	76	195	7.73	733	3.35	8.26		
2	08/06/06	3.0	4	11	D	0	48	113	80	176	7.73	733	3.35	8.26		
2	08/06/06	3.0	4	11	D	0	45	124	85	173	7.67	738	1.53	8.27		
2	08/06/06	3.0	4	11	D	0	42	117	79	177	7.67	738	1.53	8.27		
2	08/06/06	3.0	4	11	D	0	42	121	80	180	7.67	738	1.53	8.27		
2	08/06/06	1.0	4	4	D	0	41	118	80	175	8.93	741	1.25	8.08		
2	08/06/06	1.0	4	4	D	0	52	141	83	196	8.93	741	1.25	8.08		
2	08/06/06	1.0	4	4	D	0	44	119	80	178	8.93	741	1.25	8.08		
2	08/06/06	2.0	4	4	D	0	44	121	82	197	8.81	740	1.79	8.11		
2	08/06/06	2.0	4	4	D	0	49	120	79	194	8.81	740	1.79	8.11		
2	08/06/06	2.0	4	4	D	0	48	123	77	183	8.81	740	1.79	8.11		
2	08/06/06	3.0	4	4	D	0	51	126	78	175	7.9	725	1.30	8.27		
2	08/06/06	3.0	4	4	D	0	52	139	82	185	7.9	725	1.30	8.27		
2	08/06/06	3.0	4	4	D	0	49	133	82	185	7.9	725	1.30	8.27		
2	08/06/06	3.0	4	4	D	0	52	121	82	184	7.64	725	1.43	8.3		
2	08/06/06	3.0	4	4	D	0	48	118	79	192	7.64	725	1.43	8.3		
2	08/06/06	3.0	4	4	D	0	50	122	79	190	7.64	725	1.43	8.3		
2	09/06/06	1.0	5	20	D	0	42	115	90	199	6.1	747	5.54	7.82	49	89
2	09/06/06	1.0	5	20	D	0	51	97	94	200	6.1	747	5.54	7.82	49	89
2	09/06/06	1.0	5	20	D	0	45	121	87	202	6.1	747	5.54	7.82	49	89
2	09/06/06	2.0	5	20	D	0	44	108	89	202	7.09	739	5.20	7.92	49	52
2	09/06/06	2.0	5	20	D	0	43	109	91	203	7.09	739	5.20	7.92	49	52
2	09/06/06	2.0	5	20	D	0	47	114	90	207	7.09	739	5.20	7.92	49	52
2	09/06/06	3.0	5	20	D	0	44	110	83	197	5.58	744	5.27	7.9		3
2	09/06/06	3.0	5	20	D	0	51	137	84	217	5.58	744	5.27	7.9		3
2	09/06/06	3.0	5	20	D	0	47	112	86	214	5.58	744	5.27	7.9		3
2	09/06/06	3.0	5	20	D	0	51	132	86	208	6.03	747	5.35	7.96		3
2	09/06/06	3.0	5	20	D	0	47	131	86	209	6.03	747	5.35	7.96		3
2	09/06/06	3.0	5	20	D	0	45	111	86	207	6.03	747	5.35	7.96		3
2	09/06/06	1.0	5	11	D	0	45	115	84	197	8.01	741	5.06	8.01		23
2	09/06/06	1.0	5	11	D	0	42	108	82	179	8.01	741	5.06	8.01		23
2	09/06/06	1.0	5	11	D	0	45	114	85	179	8.01	741	5.06	8.01		23
2	09/06/06	2.0	5	11	D	0	50	114	79	188	8.03	742	5.11	8.05		5
2	09/06/06	2.0	5	11	D	0	44	120	81	174	8.03	742	5.11	8.05		5
2	09/06/06	2.0	5	11	D	0	60	113	81	186	8.03	742	5.11	8.05		5
2	09/06/06	3.0	5	11	D	0	48	125	80	174	6.89	741	4.29	8.2		2
2	09/06/06	3.0	5	11	D	0	48	128	80	180	6.89	741	4.29	8.2		2
2	09/06/06	3.0	5	11	D	0	45	138	82	189	6.89	741	4.29	8.2		2
2	09/06/06	3.0	5	11	D	0	42	120	76	183	7.4	743	4.70	8.19		2
2	09/06/06	3.0	5	11	D	0	50	122	83	179	7.4	743	4.70	8.19		2
2	09/06/06	3.0	5	11	D	0	46	117	83	177	7.4	743	4.70	8.19		2
2	09/06/06	1.0	5	4	D	0	54	112	81	192	8.52	742	4.94	8.04		
2	09/06/06	1.0	5	4	D	0	51	127	82	185	8.52	742	4.94	8.04		
2	09/06/06	1.0	5	4	D	0	51	122	84	179	8.52	742	4.94	8.04		
2	09/06/06	2.0	5	4	D	0	48	120	81	199	8.4	741	5.05	8.09		
2	09/06/06	2.0	5	4	D	0	50	123	81	185	8.4	741	5.05	8.09		
2	09/06/06	2.0	5	4	D	0	51	111	81	183	8.4	741	5.05	8.09		
2	09/06/06	3.0	5	4	D	0	44	132	80	174	7.35	743	4.55	8.27		
2	09/06/06	3.0	5	4	D	0	48	128	80	188	7.35	743	4.55	8.27		
2	09/06/06	3.0	5	4	D	0	46	127	79	191	7.35	743	4.55	8.27		
2	09/06/06	3.0	5	4	D	0	51	135	79	182	7.36	733	4.66	8.27		
2	09/06/06	3.0	5	4	D	0	53	119	84	183	7.36	733	4.66	8.27		
2	09/06/06	3.0	5	4	D	0	47	122	82	184	7.36	733	4.66	8.27		
2	16/06/06	1.0	12	20	D	0	47	88	102	237	5.16	755	4.78	7.52	42	193
2	16/06/06	1.0	12	20	D	0	54	96	101	239	5.16	755	4.78	7.52	42	193
2	16/06/06	1.0	12	20	D	0	54	110	105	227	5.16	755	4.78	7.52	42	193
2	16/06/06	2.0	12	20	D	0	49	86	94	222	7.06	756	4.25	7.68	49	112
2	16/06/06	2.0	12	20	D	0	50	85	102	229	7.06	756	4.25	7.68	49	112
2	16/06/06	2.0	12	20	D	0	54	100	102	220	7.06	756	4.25	7.68	49	112
2	16/06/06	3.0	12	20	D	0	53	112	98	238	6.21	746	6.04	7.53	3	7
2	16/06/06	3.0	12	20	D	0	48	106	97	224	6.21	746	6.04	7.53	3	7
2	16/06/06	3.0	12	20	D	0	46	108	103	248	6.21	746	6.04	7.53	3	7
2	16/06/06	3.0	12	20	D	0	51	96	100	255	5.95	749	6.32	7.54	3	7
2	16/06/06	3.0	12	20	D	0	45	96	100	231	5.95	749	6.32	7.54	3	7

2	16/06/06	3.0	12	20	D	0	45	97	101	240	5.95	749	6.32	7.54	3	7
2	16/06/06	1.0	12	11	D	0	49	91	84	197	7.87	744	3.72	7.9	82	93
2	16/06/06	1.0	12	11	D	0	42	100	90	197	7.87	744	3.72	7.9	82	93
2	16/06/06	1.0	12	11	D	0	45	85	89	201	7.87	744	3.72	7.9	82	93
2	16/06/06	2.0	12	11	D	0	48	90	90	203	7.75	747	4.06	7.92	41	32
2	16/06/06	2.0	12	11	D	0	41	90	85	214	7.75	747	4.06	7.92	41	32
2	16/06/06	2.0	12	11	D	0	49	85	89	199	7.75	747	4.06	7.92	41	32
2	16/06/06	3.0	12	11	D	0	44	115	87	193	7.43	741	5.25	8.07	3	5
2	16/06/06	3.0	12	11	D	0	37	95	90	207	7.43	741	5.25	8.07	3	5
2	16/06/06	3.0	12	11	D	0	46	115	85	197	7.43	741	5.25	8.07	3	5
2	16/06/06	3.0	12	11	D	0	50	110	85	189	7.2	747	4.51	8.07	3	5
2	16/06/06	3.0	12	11	D	0	41	119	85	202	7.2	747	4.51	8.07	3	5
2	16/06/06	3.0	12	11	D	0	51	116	87	208	7.2	747	4.51	8.07	3	5
2	16/06/06	1.0	12	4	D	0	49	104	85	196	8.75	735	4.05	8.01	3	42
2	16/06/06	1.0	12	4	D	0	42	97	86	183	8.75	735	4.05	8.01	3	42
2	16/06/06	1.0	12	4	D	0	46	117	87	202	8.75	735	4.05	8.01	3	42
2	16/06/06	2.0	12	4	D	0	44	118	88	189	8.9	738	3.89	8.03	17	10
2	16/06/06	2.0	12	4	D	0	50	131	89	188	8.9	738	3.89	8.03	17	10
2	16/06/06	2.0	12	4	D	0	51	107	83	200	8.9	738	3.89	8.03	17	10
2	16/06/06	3.0	12	4	D	0	45	108	81	192	8.03	741	4.95	8.17		
2	16/06/06	3.0	12	4	D	0	47	91	82	185	8.03	741	4.95	8.17		
2	16/06/06	3.0	12	4	D	0	51	116	79	198	8.03	741	4.95	8.17		
2	16/06/06	3.0	12	4	D	0	46	138	81	187	8.31	738	4.22	8.2		
2	16/06/06	3.0	12	4	D	0	46	139	81	193	8.31	738	4.22	8.2		
2	16/06/06	3.0	12	4	D	0	46	100	82	187	8.31	738	4.22	8.2		
2	23/06/06	1.0	19	20	D	0	46	109	98	233	6.17	743	5.21	7.41	86	188
2	23/06/06	1.0	19	20	D	0	47	93	102	223	6.17	743	5.21	7.41	86	188
2	23/06/06	1.0	19	20	D	0	45	97	97	216	6.17	743	5.21	7.41	86	188
2	23/06/06	2.0	19	20	D	0	52	92	103	223	6.32	736	5.13	7.42	47	154
2	23/06/06	2.0	19	20	D	0	55	95	102	239	6.32	736	5.13	7.42	47	154
2	23/06/06	2.0	19	20	D	0	50	88	101	231	6.32	736	5.13	7.42	47	154
2	23/06/06	3.0	19	20	D	0	50	95	99	222	6.57	748	5.37	7.45	3	6
2	23/06/06	3.0	19	20	D	0	51	92	96	222	6.57	748	5.37	7.45	3	6
2	23/06/06	3.0	19	20	D	0	47	97	97	222	6.57	748	5.37	7.45	3	6
2	23/06/06	3.0	19	20	D	0	61	116	105	264	6.56	749	5.83	7.47	3	6
2	23/06/06	3.0	19	20	D	0	55	110	108	260	6.56	749	5.83	7.47	3	6
2	23/06/06	3.0	19	20	D	0	62	120	108	255	6.56	749	5.83	7.47	3	6
2	23/06/06	1.0	19	11	D	0	43	60	89	212	7.84	746	4.18	7.81	73	93
2	23/06/06	1.0	19	11	D	0	46	68	88	211	7.84	746	4.18	7.81	73	93
2	23/06/06	1.0	19	11	D	0	44	79	87	198	7.84	746	4.18	7.81	73	93
2	23/06/06	2.0	19	11	D	0	45	79	89	208	8.12	748	3.68	7.91	37	27
2	23/06/06	2.0	19	11	D	0	45	97	95	207	8.12	748	3.68	7.91	37	27
2	23/06/06	2.0	19	11	D	0	42	76	95	206	8.12	748	3.68	7.91	37	27
2	23/06/06	3.0	19	11	D	0	49	124	90	205	7.95	744	4.16	8.09	3	8
2	23/06/06	3.0	19	11	D	0	53	125	87	220	7.95	744	4.16	8.09	3	8
2	23/06/06	3.0	19	11	D	0	49	132	88	205	7.95	744	4.16	8.09	3	8
2	23/06/06	3.0	19	11	D	0	45	110	88	195	7.91	746	4.13	8.03	3	8
2	23/06/06	3.0	19	11	D	0	46	120	92	203	7.91	746	4.13	8.03	3	8
2	23/06/06	3.0	19	11	D	0	50	103	88	202	7.91	746	4.13	8.03	3	8
2	23/06/06	1.0	19	4	D	0	49	90	84	181	8.44	738	4.11	7.97	5	79
2	23/06/06	1.0	19	4	D	0	48	90	92	202	8.44	738	4.11	7.97	5	79
2	23/06/06	1.0	19	4	D	0	50	90	90	187	8.44	738	4.11	7.97	5	79
2	23/06/06	2.0	19	4	D	0	45	90	86	195	8.6	743	4.14	8	30	11
2	23/06/06	2.0	19	4	D	0	44	91	88	193	8.6	743	4.14	8	30	11
2	23/06/06	2.0	19	4	D	0	50	104	85	199	8.6	743	4.14	8	30	11
2	23/06/06	3.0	19	4	D	0	44	115	82	204	8.61	732	4.00	8.12		
2	23/06/06	3.0	19	4	D	0	48	106	82	199	8.61	732	4.00	8.12		
2	23/06/06	3.0	19	4	D	0	50	118	84	200	8.61	732	4.00	8.12		
2	23/06/06	3.0	19	4	D	0	50	121	79	181	8.03	744	3.59	8.19		
2	23/06/06	3.0	19	4	D	0	49	116	84	192	8.03	744	3.59	8.19		
2	23/06/06	3.0	19	4	D	0	50	104	96	179	8.03	744	3.59	8.19		
2	30/06/06	1.0	26	20	D	0	39	94	102	232	6.9	745	5.86	7.46	86	203
2	30/06/06	1.0	26	20	D	0	44	95	107	253	6.9	745	5.86	7.46	86	203
2	30/06/06	1.0	26	20	D	0	50	88	100	248	6.9	745	5.86	7.46	86	203
2	30/06/06	2.0	26	20	D	0	50	86	102	226	6.78	751	5.13	7.44	51	157
2	30/06/06	2.0	26	20	D	0	52	93	105	242	6.78	751	5.13	7.44	51	157
2	30/06/06	2.0	26	20	D	0	45	92	106	235	6.78	751	5.13	7.44	51	157
2	30/06/06	3.0	26	20	D	0	49	116	104	237	7.1	744	5.76	7.48	3	7
2	30/06/06	3.0	26	20	D	0	59	126	100	254	7.1	744	5.76	7.48	3	7
2	30/06/06	3.0	26	20	D	0	55	119	102	237	7.1	744	5.76	7.48	3	7
2	30/06/06	3.0	26	20	D	0	63	129	97	221	6.85	748	5.56	7.45	3	7
2	30/06/06	3.0	26	20	D	0	57	134	101	218	6.85	748	5.56	7.45	3	7
2	30/06/06	3.0	26	20	D	0	57	130	103	227	6.85	748	5.56	7.45	3	7
2	30/06/06	1.0	26	11	D	0	41	63	92	202	7.73	749	4.26	7.85	77	110
2	30/06/06	1.0	26	11	D	0	44	65	88	219	7.73	749	4.26	7.85	77	110

2	30/06/06	1.0	26	11	D	0	45	69	87	201	7.73	749	4.26	7.85	77	110
2	30/06/06	2.0	26	11	D	0	45	83	92	198	7.7	748	4.84	7.88	28	27
2	30/06/06	2.0	26	11	D	0	51	88	93	216	7.7	748	4.84	7.88	28	27
2	30/06/06	2.0	26	11	D	0	42	84	91	236	7.7	748	4.84	7.88	28	27
2	30/06/06	3.0	26	11	D	0	41	65	90	207	7.71	737	4.22	8.06	3	7
2	30/06/06	3.0	26	11	D	0	37	83	94	223	7.71	737	4.22	8.06	3	7
2	30/06/06	3.0	26	11	D	0	38	83	92	217	7.71	737	4.22	8.06	3	7
2	30/06/06	3.0	26	11	D	0	46	83	88	200	7.78	744	4.56	8.08	3	7
2	30/06/06	3.0	26	11	D	0	41	92	91	197	7.78	744	4.56	8.08	3	7
2	30/06/06	3.0	26	11	D	0	46	82	88	200	7.78	744	4.56	8.08	3	7
2	30/06/06	1.0	26	4	D	0	48	74	87	197	9.45	743	4.44	8.02	5	74
2	30/06/06	1.0	26	4	D	0	41	66	87	202	9.45	743	4.44	8.02	5	74
2	30/06/06	1.0	26	4	D	0	40	63	85	197	9.45	743	4.44	8.02	5	74
2	30/06/06	2.0	26	4	D	0	43	70	89	186	8.78	749	4.44	8.06	25	8
2	30/06/06	2.0	26	4	D	0	44	93	88	200	8.78	749	4.44	8.06	25	8
2	30/06/06	2.0	26	4	D	0	40	77	92	201	8.78	749	4.44	8.06	25	8
2	30/06/06	3.0	26	4	D	0	50	97	88	190	9.2	741	4.48	8.25	1	5
2	30/06/06	3.0	26	4	D	0	55	96	86	190	9.2	741	4.48	8.25	1	5
2	30/06/06	3.0	26	4	D	0	42	99	83	192	9.2	741	4.48	8.25	1	5
2	30/06/06	3.0	26	4	D	0	56	115	82	206	8.42	740	4.24	8.22	1	5
2	30/06/06	3.0	26	4	D	0	50	92	92	198	8.42	740	4.24	8.22	1	5
2	30/06/06	3.0	26	4	D	0	46	108	87	195	8.42	740	4.24	8.22	1	5
3	03/07/06	1.0	1	4	D	0	68	197	95	240	10.4	615	5.54	7.91		
3	03/07/06	1.0	1	4	D	0	74	208	97	260	10.4	615	5.54	7.91		
3	03/07/06	1.0	1	4	D	0	73	208	95	259	10.4	615	5.54	7.91		
3	03/07/06	2.0	1	4	D	0	85	196	101	242	10.29	616	5.16	7.93		
3	03/07/06	2.0	1	4	D	0	82	206	103	254	10.29	616	5.16	7.93		
3	03/07/06	2.0	1	4	D	0	79	218	97	254	10.29	616	5.16	7.93		
3	03/07/06	3.0	1	4	D	0	77	191	98	244	9.59	617	3.68	8.08		
3	03/07/06	3.0	1	4	D	0	72	194	100	240	9.59	617	3.68	8.08		
3	03/07/06	3.0	1	4	D	0	74	196	101	245	9.59	617	3.68	8.08		
3	03/07/06	1.0	1	11	D	0	68	197	95	240	10.4	615	5.54	7.91		
3	03/07/06	1.0	1	11	D	0	74	208	97	260	10.4	615	5.54	7.91		
3	03/07/06	1.0	1	11	D	0	73	208	95	259	10.4	615	5.54	7.91		
3	03/07/06	2.0	1	11	D	0	85	196	101	242	10.29	616	5.16	7.93		
3	03/07/06	2.0	1	11	D	0	82	206	103	254	10.29	616	5.16	7.93		
3	03/07/06	2.0	1	11	D	0	79	218	97	254	10.29	616	5.16	7.93		
3	03/07/06	3.0	1	11	D	0	77	191	98	244	9.59	617	3.68	8.08		
3	03/07/06	3.0	1	11	D	0	72	194	100	240	9.59	617	3.68	8.08		
3	03/07/06	3.0	1	11	D	0	74	196	101	245	9.59	617	3.68	8.08		
3	03/07/06	1.0	1	20	D	0	68	197	95	240	10.4	615	5.54	7.91		
3	03/07/06	1.0	1	20	D	0	74	208	97	260	10.4	615	5.54	7.91		
3	03/07/06	1.0	1	20	D	0	73	208	95	259	10.4	615	5.54	7.91		
3	03/07/06	2.0	1	20	D	0	85	196	101	242	10.29	616	5.16	7.93		
3	03/07/06	2.0	1	20	D	0	82	206	103	254	10.29	616	5.16	7.93		
3	03/07/06	2.0	1	20	D	0	79	218	97	254	10.29	616	5.16	7.93		
3	03/07/06	3.0	1	20	D	0	77	191	98	244	9.59	617	3.68	8.08		
3	03/07/06	3.0	1	20	D	0	72	194	100	240	9.59	617	3.68	8.08		
3	03/07/06	3.0	1	20	D	0	74	196	101	245	9.59	617	3.68	8.08		
3	04/07/06	1.0	2	20	D	0	84	190	101	272	7.7	605	3.44	7.95		
3	04/07/06	1.0	2	20	D	0	81	198	102	276	7.7	605	3.44	7.95		
3	04/07/06	1.0	2	20	D	0	83	201	107	275	7.7	605	3.44	7.95		
3	04/07/06	2.0	2	20	D	0	82	201	101	269	7.77	606	4.58	7.95		
3	04/07/06	2.0	2	20	D	0	78	196	104	268	7.77	606	4.58	7.95		
3	04/07/06	2.0	2	20	D	0	78	191	102	287	7.77	606	4.58	7.95		
3	04/07/06	2.0	2	20	D	0	78	196	104	273	7.24	608	4.32	7.88		
3	04/07/06	2.0	2	20	D	0	82	204	102	270	7.24	608	4.32	7.88		
3	04/07/06	2.0	2	20	D	0	76	212	103	265	7.24	608	4.32	7.88		
3	04/07/06	3.0	2	20	D	0	86	212	107	261	6.63	614	4.19	8.15		
3	04/07/06	3.0	2	20	D	0	80	195	101	263	6.63	614	4.19	8.15		
3	04/07/06	3.0	2	20	D	0	70	206	105	270	6.63	614	4.19	8.15		
3	04/07/06	1.0	2	11	D	0	71	190	101	263	8.03	609	3.95	7.92		
3	04/07/06	1.0	2	11	D	0	78	197	104	265	8.03	609	3.95	7.92		
3	04/07/06	1.0	2	11	D	0	78	214	107	268	8.03	609	3.95	7.92		
3	04/07/06	2.0	2	11	D	0	81	184	98	248	7.79	603	4.37	7.96		
3	04/07/06	2.0	2	11	D	0	82	202	100	252	7.79	603	4.37	7.96		
3	04/07/06	2.0	2	11	D	0	75	206	101	251	7.79	603	4.37	7.96		
3	04/07/06	2.0	2	11	D	0	91	204	104	288	7.82	606	3.28	7.97		
3	04/07/06	2.0	2	11	D	0	87	192	107	289	7.82	606	3.28	7.97		
3	04/07/06	2.0	2	11	D	0	78	194	108	290	7.82	606	3.28	7.97		
3	04/07/06	3.0	2	11	D	0	74	189	104	244	6.77	604	3.54	8.18		
3	04/07/06	3.0	2	11	D	0	70	176	109	265	6.77	604	3.54	8.18		
3	04/07/06	3.0	2	11	D	0	82	190	100	252	6.77	604	3.54	8.18		
3	04/07/06	1.0	2	4	D	0	78	192	104	289	8	609	3.84	7.9		
3	04/07/06	1.0	2	4	D	0	86	191	103	264	8	609	3.84	7.9		

3	04/07/06	1.0	2	4	D	0	78	198	104	257	8	609	3.84	7.9		
3	04/07/06	2.0	2	4	D	0	80	195	100	250	7.86	612	4.06	7.98		
3	04/07/06	2.0	2	4	D	0	74	200	105	255	7.86	612	4.06	7.98		
3	04/07/06	2.0	2	4	D	0	79	197	102	270	7.86	612	4.06	7.98		
3	04/07/06	2.0	2	4	D	0							0.00			
3	04/07/06	2.0	2	4	D	0							0.00			
3	04/07/06	2.0	2	4	D	0							0.00			
3	04/07/06	3.0	2	4	D	0	78	199	105	251	7.29	606	3.99	8.18		
3	04/07/06	3.0	2	4	D	0	72	193	103	273	7.29	606	3.99	8.18		
3	04/07/06	3.0	2	4	D	0	82	189	102	257	7.29	606	3.99	8.18		
3	05/07/06	1.0	3	20	D	0	91	189	106	269	6.48	609	3.63	7.81	10	2
3	05/07/06	1.0	3	20	D	0	75	201	110	270	6.48	609	3.63	7.81	10	2
3	05/07/06	1.0	3	20	D	0	77	205	113	286	6.48	609	3.63	7.81	10	2
3	05/07/06	2.0	3	20	D	0	78	205	106	275	7.61	604	3.42	7.97		1
3	05/07/06	2.0	3	20	D	0	82	205	105	269	7.61	604	3.42	7.97		1
3	05/07/06	2.0	3	20	D	0	71	195	106	270	7.61	604	3.42	7.97		1
3	05/07/06	2.0	3	20	D	0	81	200	101	262	7.49	606	2.96	7.95		1
3	05/07/06	2.0	3	20	D	0	79	194	105	278	7.49	606	2.96	7.95		1
3	05/07/06	2.0	3	20	D	0	80	202	104	274	7.49	606	2.96	7.95		1
3	05/07/06	3.0	3	20	D	0	80	185	106	275	5.52	605	4.39	8.08		
3	05/07/06	3.0	3	20	D	0	83	202	102	276	5.52	605	4.39	8.08		
3	05/07/06	3.0	3	20	D	0	83	197	101	282	5.52	605	4.39	8.08		
3	05/07/06	1.0	3	11	D	0	77	225	104	258	7.1	610	3.20	7.82		
3	05/07/06	1.0	3	11	D	0	79	206	104	269	7.1	610	3.20	7.82		
3	05/07/06	1.0	3	11	D	0	74	211	106	255	7.1	610	3.20	7.82		
3	05/07/06	2.0	3	11	D	0	77	193	102	257	7.67	613	3.27	7.99		
3	05/07/06	2.0	3	11	D	0	74	192	104	265	7.67	613	3.27	7.99		
3	05/07/06	2.0	3	11	D	0	78	187	104	273	7.67	613	3.27	7.99		
3	05/07/06	2.0	3	11	D	0	76	191	104	266	7.67	610	3.01	7.98		
3	05/07/06	2.0	3	11	D	0	83	203	105	261	7.67	610	3.01	7.98		
3	05/07/06	2.0	3	11	D	0	75	207	106	275	7.67	610	3.01	7.98		
3	05/07/06	3.0	3	11	D	0	74	178	98	257	6.53	606	2.99	8.2		
3	05/07/06	3.0	3	11	D	0	78	194	104	269	6.53	606	2.99	8.2		
3	05/07/06	3.0	3	11	D	0	78	180	104	261	6.53	606	2.99	8.2		
3	05/07/06	1.0	3	4	D	0	81	202	108	253	7.73	610	3.14	7.89		
3	05/07/06	1.0	3	4	D	0	79	187	104	289	7.73	610	3.14	7.89		
3	05/07/06	1.0	3	4	D	0	88	212	104	267	7.73	610	3.14	7.89		
3	05/07/06	2.0	3	4	D	0	74	194	100	261	7.55	610	3.40	7.97		
3	05/07/06	2.0	3	4	D	0	73	206	100	257	7.55	610	3.40	7.97		
3	05/07/06	2.0	3	4	D	0	83	208	104	271	7.55	610	3.40	7.97		
3	05/07/06	2.0	3	4	D	0	77	197	106	257	7.38	612	3.28	7.98		
3	05/07/06	2.0	3	4	D	0	80	194	104	258	7.38	612	3.28	7.98		
3	05/07/06	2.0	3	4	D	0	73	193	104	256	7.38	612	3.28	7.98		
3	05/07/06	3.0	3	4	D	0	82	173	101	252	6.45	606	2.77	8.19		
3	05/07/06	3.0	3	4	D	0	78	185	100	268	6.45	606	2.77	8.19		
3	05/07/06	3.0	3	4	D	0	78	198	104	260	6.45	606	2.77	8.19		
3	06/07/06	1.0	4	20	D	0	73	190	109	271	7.15	601	3.39	7.9	24	37
3	06/07/06	1.0	4	20	D	0	83	194	106	282	7.15	601	3.39	7.9	24	37
3	06/07/06	1.0	4	20	D	0	79	188	115	272	7.15	601	3.39	7.9	24	37
3	06/07/06	2.0	4	20	D	0	81	194	104	274	7.48	603	3.35	7.94	5	28
3	06/07/06	2.0	4	20	D	0	81	192	110	267	7.48	603	3.35	7.94	5	28
3	06/07/06	2.0	4	20	D	0	82	214	106	276	7.48	603	3.35	7.94	5	28
3	06/07/06	2.0	4	20	D	0	87	203	105	280	5.86	597	4.10	7.77	5	28
3	06/07/06	2.0	4	20	D	0	84	213	112	271	5.86	597	4.10	7.77	5	28
3	06/07/06	2.0	4	20	D	0	87	223	105	278	5.86	597	4.10	7.77	5	28
3	06/07/06	3.0	4	20	D	0	80	191	101	271	6.66	597	3.83	8.16	5	4
3	06/07/06	3.0	4	20	D	0	75	194	105	265	6.66	597	3.83	8.16	5	4
3	06/07/06	3.0	4	20	D	0	73	197	103	279	6.66	597	3.83	8.16	5	4
3	06/07/06	1.0	4	11	D	0	82	195	108	265	6.84	599	3.73	7.83		
3	06/07/06	1.0	4	11	D	0	76	193	102	281	6.84	599	3.73	7.83		
3	06/07/06	1.0	4	11	D	0	76	213	113	288	6.84	599	3.73	7.83		
3	06/07/06	2.0	4	11	D	0	70	192	111	254	7.64	599	3.25	7.94		
3	06/07/06	2.0	4	11	D	0	68	200	105	259	7.64	599	3.25	7.94		
3	06/07/06	2.0	4	11	D	0	81	204	100	258	7.64	599	3.25	7.94		
3	06/07/06	2.0	4	11	D	0	76	201	106	260	7.4	604	3.61	7.94		
3	06/07/06	2.0	4	11	D	0	80	209	108	260	7.4	604	3.61	7.94		
3	06/07/06	2.0	4	11	D	0	84	192	101	255	7.4	604	3.61	7.94		
3	06/07/06	3.0	4	11	D	0	72	189	100	249	6.3	604	2.85	8.12		
3	06/07/06	3.0	4	11	D	0	73	185	101	249	6.3	604	2.85	8.12		
3	06/07/06	3.0	4	11	D	0	85	186	105	258	6.3	604	2.85	8.12		
3	06/07/06	1.0	4	4	D	0	81	203	105	262	7.56	602	3.67	7.92		
3	06/07/06	1.0	4	4	D	0	73	203	107	258	7.56	602	3.67	7.92		
3	06/07/06	1.0	4	4	D	0	75	206	101	261	7.56	602	3.67	7.92		
3	06/07/06	2.0	4	4	D	0	85	202	102	256	7.68	600	3.39	8		
3	06/07/06	2.0	4	4	D	0	77	205	100	263	7.68	600	3.39	8		

3	06/07/06	2.0	4	4	D	0	82	206	103	258	7.68	600	3.39	8		
3	06/07/06	2.0	4	4	D	0	76	207	103	271	7.67	605	3.60	7.96		
3	06/07/06	2.0	4	4	D	0	83	202	105	282	7.67	605	3.60	7.96		
3	06/07/06	2.0	4	4	D	0	76	201	100	275	7.67	605	3.60	7.96		
3	06/07/06	3.0	4	4	D	0	79	200	101	248	7.07	595	3.02	8.14		
3	06/07/06	3.0	4	4	D	0	73	193	102	264	7.07	595	3.02	8.14		
3	06/07/06	3.0	4	4	D	0	79	198	100	267	7.07	595	3.02	8.14		
3	07/07/06	1.0	5	20	D	0	79	196	111	281	7.73	606	3.55	8.07	75	108
3	07/07/06	1.0	5	20	D	0	76	198	111	302	7.73	606	3.55	8.07	75	108
3	07/07/06	1.0	5	20	D	0	89	189	111	301	7.73	606	3.55	8.07	75	108
3	07/07/06	2.0	5	20	D	0	88	186	117	266	6.2	592	4.13	7.88	10	96
3	07/07/06	2.0	5	20	D	0	86	187	109	271	6.2	592	4.13	7.88	10	96
3	07/07/06	2.0	5	20	D	0	83	187	112	297	6.2	592	4.13	7.88	10	96
3	07/07/06	2.0	5	20	D	0	82	203	105	292	7.9	604	3.43	8.13	10	96
3	07/07/06	2.0	5	20	D	0	77	190	111	279	7.9	604	3.43	8.13	10	96
3	07/07/06	2.0	5	20	D	0	76	202	106	299	7.9	604	3.43	8.13	10	96
3	07/07/06	3.0	5	20	D	0	74	192	109	286	7.42	600	3.05	8.37	5	7
3	07/07/06	3.0	5	20	D	0	76	197	105	271	7.42	600	3.05	8.37	5	7
3	07/07/06	3.0	5	20	D	0	76	221	107	275	7.42	600	3.05	8.37	5	7
3	07/07/06	1.0	5	11	D	0	82	189	106	262	7.98	601	4.11	8.06	4	7
3	07/07/06	1.0	5	11	D	0	70	190	103	269	7.98	601	4.11	8.06	4	7
3	07/07/06	1.0	5	11	D	0	80	187	108	281	7.98	601	4.11	8.06	4	7
3	07/07/06	2.0	5	11	D	0	82	202	105	254	7.6	601	3.66	8.1		8
3	07/07/06	2.0	5	11	D	0	80	197	106	251	7.6	601	3.66	8.1		8
3	07/07/06	2.0	5	11	D	0	85	210	106	262	7.6	601	3.66	8.1		8
3	07/07/06	2.0	5	11	D	0	76	201	105	244	8.46	602	3.41	8.18		8
3	07/07/06	2.0	5	11	D	0	77	188	102	268	8.46	602	3.41	8.18		8
3	07/07/06	2.0	5	11	D	0	78	197	102	265	8.46	602	3.41	8.18		8
3	07/07/06	3.0	5	11	D	0	75	194	101	258	7.33	599	2.71	8.43		4
3	07/07/06	3.0	5	11	D	0	80	194	101	254	7.33	599	2.71	8.43		4
3	07/07/06	3.0	5	11	D	0	76	193	102	258	7.33	599	2.71	8.43		4
3	07/07/06	1.0	5	4	D	0	80	201	105	259	8.33	599	3.56	8.13		
3	07/07/06	1.0	5	4	D	0	80	204	102	280	8.33	599	3.56	8.13		
3	07/07/06	1.0	5	4	D	0	80	212	105	280	8.33	599	3.56	8.13		
3	07/07/06	2.0	5	4	D	0	75	205	102	258	8.19	604	3.35	8.18		
3	07/07/06	2.0	5	4	D	0	77	199	102	260	8.19	604	3.35	8.18		
3	07/07/06	2.0	5	4	D	0	77	199	104	254	8.19	604	3.35	8.18		
3	07/07/06	2.0	5	4	D	0	76	195	101	258	8.5	606	3.44	8.18		
3	07/07/06	2.0	5	4	D	0	75	191	104	266	8.5	606	3.44	8.18		
3	07/07/06	2.0	5	4	D	0	78	202	101	272	8.5	606	3.44	8.18		
3	07/07/06	3.0	5	4	D	0	90	210	113	265	7.85	609	3.85	8.4		
3	07/07/06	3.0	5	4	D	0	81	215	107	279	7.85	609	3.85	8.4		
3	07/07/06	3.0	5	4	D	0	77	210	114	269	7.85	609	3.85	8.4		
3	14/07/06	1.0	12	20	D	0	81	222	110	309	5.16	755	3.97	7.52	84	164
3	14/07/06	1.0	12	20	D	0	78	197	112	290	5.16	755	3.97	7.52	84	164
3	14/07/06	1.0	12	20	D	0	74	184	111	288	5.16	755	3.97	7.52	84	164
3	14/07/06	2.0	12	20	D	0	78	206	107	297	7.06	756	5.87	7.68	17	199
3	14/07/06	2.0	12	20	D	0	81	203	113	302	7.06	756	5.87	7.68	17	199
3	14/07/06	2.0	12	20	D	0	82	209	110	302	7.06	756	5.87	7.68	17	199
3	14/07/06	2.0	12	20	D	0	75	195	119	322	6.21	746	3.76	7.53	17	199
3	14/07/06	2.0	12	20	D	0	77	186	117	330	6.21	746	3.76	7.53	17	199
3	14/07/06	2.0	12	20	D	0	75	197	117	341	6.21	746	3.76	7.53	17	199
3	14/07/06	3.0	12	20	D	0	78	194	116	294	5.95	749	4.30	7.54	10	23
3	14/07/06	3.0	12	20	D	0	77	194	110	284	5.95	749	4.30	7.54	10	23
3	14/07/06	3.0	12	20	D	0	80	187	111	294	5.95	749	4.30	7.54	10	23
3	14/07/06	1.0	12	11	D	0	78	164	104	260	7.87	744	4.69	7.9	9	29
3	14/07/06	1.0	12	11	D	0	73	184	107	278	7.87	744	4.69	7.9	9	29
3	14/07/06	1.0	12	11	D	0	79	189	107	275	7.87	744	4.69	7.9	9	29
3	14/07/06	2.0	12	11	D	0	80	192	102	270	7.75	747	5.09	7.92	2	18
3	14/07/06	2.0	12	11	D	0	76	190	111	259	7.75	747	5.09	7.92	2	18
3	14/07/06	2.0	12	11	D	0	78	197	113	259	7.75	747	5.09	7.92	2	18
3	14/07/06	2.0	12	11	D	0	78	183	106	255	7.43	741	4.54	8.07	2	18
3	14/07/06	2.0	12	11	D	0	75	194	111	266	7.43	741	4.54	8.07	2	18
3	14/07/06	2.0	12	11	D	0	78	186	106	272	7.43	741	4.54	8.07	2	18
3	14/07/06	3.0	12	11	D	0	80	191	105	272	7.2	747	4.88	8.07		7
3	14/07/06	3.0	12	11	D	0	74	194	107	274	7.2	747	4.88	8.07		7
3	14/07/06	3.0	12	11	D	0	81	194	110	270	7.2	747	4.88	8.07		7
3	14/07/06	1.0	12	4	D	0	80	196	106	270	8.75	735	4.01	8.01	7	6
3	14/07/06	1.0	12	4	D	0	77	192	105	276	8.75	735	4.01	8.01	7	6
3	14/07/06	1.0	12	4	D	0	79	191	114	276	8.75	735	4.01	8.01	7	6
3	14/07/06	2.0	12	4	D	0	77	206	104	261	8.9	738	4.79	8.03		4
3	14/07/06	2.0	12	4	D	0	78	206	101	268	8.9	738	4.79	8.03		4
3	14/07/06	2.0	12	4	D	0	79	195	106	337	8.9	738	4.79	8.03		4
3	14/07/06	2.0	12	4	D	0	78	194	105	256	8.03	741	4.15	8.17		4
3	14/07/06	2.0	12	4	D	0	80	197	105	271	8.03	741	4.15	8.17		4

3	14/07/06	2.0	12	4	D	0	77	190	101	270	8.03	741	4.15	8.17		4
3	14/07/06	3.0	12	4	D	0	75	185	102	250	8.31	738	4.24	8.2	1	1
3	14/07/06	3.0	12	4	D	0	78	196	101	269	8.31	738	4.24	8.2	1	1
3	14/07/06	3.0	12	4	D	0	78	180	104	264	8.31	738	4.24	8.2	1	1
3	21/07/06	1.0	19	20	D	0	73	182	120	318	6.17	743	3.07	7.41	85	167
3	21/07/06	1.0	19	20	D	0	72	177	119	323	6.17	743	3.07	7.41	85	167
3	21/07/06	1.0	19	20	D	0	73	193	125	318	6.17	743	3.07	7.41	85	167
3	21/07/06	2.0	19	20	D	0	76	180	108	275	6.32	736	3.08	7.42	18	207
3	21/07/06	2.0	19	20	D	0	73	195	107	286	6.32	736	3.08	7.42	18	207
3	21/07/06	2.0	19	20	D	0	79	211	112	277	6.32	736	3.08	7.42	18	207
3	21/07/06	2.0	19	20	D	0	76	193	105	286	6.57	748	3.15	7.45	18	207
3	21/07/06	2.0	19	20	D	0	82	178	111	294	6.57	748	3.15	7.45	18	207
3	21/07/06	2.0	19	20	D	0	74	186	111	281	6.57	748	3.15	7.45	18	207
3	21/07/06	3.0	19	20	D	0	81	209	119	313	6.56	749	3.56	7.47	10	23
3	21/07/06	3.0	19	20	D	0	79	209	118	304	6.56	749	3.56	7.47	10	23
3	21/07/06	3.0	19	20	D	0	81	200	121	318	6.56	749	3.56	7.47	10	23
3	21/07/06	1.0	19	11	D	0	77	171	107	282	7.84	746	1.99	7.81	9	38
3	21/07/06	1.0	19	11	D	0	70	178	109	287	7.84	746	1.99	7.81	9	38
3	21/07/06	1.0	19	11	D	0	82	196	110	272	7.84	746	1.99	7.81	9	38
3	21/07/06	2.0	19	11	D	0	76	185	111	299	8.12	748	3.34	7.91	6	18
3	21/07/06	2.0	19	11	D	0	77	195	111	308	8.12	748	3.34	7.91	6	18
3	21/07/06	2.0	19	11	D	0	78	190	114	309	8.12	748	3.34	7.91	6	18
3	21/07/06	2.0	19	11	D	0	78	194	116	306	7.95	744	3.43	8.09	6	18
3	21/07/06	2.0	19	11	D	0	71	205	114	314	7.95	744	3.43	8.09	6	18
3	21/07/06	2.0	19	11	D	0	73	191	125	309	7.95	744	3.43	8.09	6	18
3	21/07/06	3.0	19	11	D	0	70	182	110	279	7.91	746	3.09	8.03		7
3	21/07/06	3.0	19	11	D	0	77	198	112	288	7.91	746	3.09	8.03		7
3	21/07/06	3.0	19	11	D	0	72	188	110	284	7.91	746	3.09	8.03		7
3	21/07/06	1.0	19	4	D	0	76	191	111	277	8.44	738	1.35	7.97	19	33
3	21/07/06	1.0	19	4	D	0	83	197	111	270	8.44	738	1.35	7.97	19	33
3	21/07/06	1.0	19	4	D	0	75	198	106	291	8.44	738	1.35	7.97	19	33
3	21/07/06	2.0	19	4	D	0	77	200	106	272	8.6	743	3.87	8	9	20
3	21/07/06	2.0	19	4	D	0	81	192	114	270	8.6	743	3.87	8	9	20
3	21/07/06	2.0	19	4	D	0	79	205	113	268	8.6	743	3.87	8	9	20
3	21/07/06	2.0	19	4	D	0	74	198	103	266	8.61	732	3.84	8.12	9	20
3	21/07/06	2.0	19	4	D	0	80	196	112	269	8.61	732	3.84	8.12	9	20
3	21/07/06	2.0	19	4	D	0	79	189	106	283	8.61	732	3.84	8.12	9	20
3	21/07/06	3.0	19	4	D	0	70	191	103	265	8.03	744	3.02	8.19	2	3
3	21/07/06	3.0	19	4	D	0	82	196	106	265	8.03	744	3.02	8.19	2	3
3	21/07/06	3.0	19	4	D	0	84	181	104	267	8.03	744	3.02	8.19	2	3
3	28/07/06	1.0	26	20	D	0	76	189	130	331	6.9	745	3.62	7.46	85	167
3	28/07/06	1.0	26	20	D	0	79	197	125	335	6.9	745	3.62	7.46	85	167
3	28/07/06	1.0	26	20	D	0	79	191	122	350	6.9	745	3.62	7.46	85	167
3	28/07/06	2.0	26	20	D	0	78	187	110	301	6.78	751	3.14	7.44	18	208
3	28/07/06	2.0	26	20	D	0	80	189	111	283	6.78	751	3.14	7.44	18	208
3	28/07/06	2.0	26	20	D	0	76	189	115	316	6.78	751	3.14	7.44	18	208
3	28/07/06	2.0	26	20	D	0	68	172	114	313	7.1	744	1.96	7.48	18	208
3	28/07/06	2.0	26	20	D	0	72	182	114	295	7.1	744	1.96	7.48	18	208
3	28/07/06	2.0	26	20	D	0	72	178	114	340	7.1	744	1.96	7.48	18	208
3	28/07/06	3.0	26	20	D	0	77	175	117	319	6.85	748	2.91	7.45	10	23
3	28/07/06	3.0	26	20	D	0	79	178	117	306	6.85	748	2.91	7.45	10	23
3	28/07/06	3.0	26	20	D	0	72	169	114	319	6.85	748	2.91	7.45	10	23
3	28/07/06	1.0	26	11	D	0	76	192	105	269	7.73	749	2.83	7.85	10	38
3	28/07/06	1.0	26	11	D	0	76	182	111	267	7.73	749	2.83	7.85	10	38
3	28/07/06	1.0	26	11	D	0	73	185	108	273	7.73	749	2.83	7.85	10	38
3	28/07/06	2.0	26	11	D	0	79	191	110	284	7.7	748	3.15	7.88	6	20
3	28/07/06	2.0	26	11	D	0	72	188	117	281	7.7	748	3.15	7.88	6	20
3	28/07/06	2.0	26	11	D	0	79	188	114	285	7.7	748	3.15	7.88	6	20
3	28/07/06	2.0	26	11	D	0	79	176	110	275	7.71	737	2.65	8.06	6	20
3	28/07/06	2.0	26	11	D	0	79	186	106	288	7.71	737	2.65	8.06	6	20
3	28/07/06	2.0	26	11	D	0	76	183	106	279	7.71	737	2.65	8.06	6	20
3	28/07/06	3.0	26	11	D	0	72	179	103	304	7.78	744	3.16	8.08		7
3	28/07/06	3.0	26	11	D	0	76	191	107	285	7.78	744	3.16	8.08		7
3	28/07/06	3.0	26	11	D	0	79	191	110	302	7.78	744	3.16	8.08		7
3	28/07/06	1.0	26	4	D	0	66	190	105	261	9.45	743	4.11	8.02	21	42
3	28/07/06	1.0	26	4	D	0	79	186	107	269	9.45	743	4.11	8.02	21	42
3	28/07/06	1.0	26	4	D	0	68	171	103	281	9.45	743	4.11	8.02	21	42
3	28/07/06	2.0	26	4	D	0	87	195	107	272	8.78	749	2.73	8.06	14	47
3	28/07/06	2.0	26	4	D	0	80	214	107	285	8.78	749	2.73	8.06	14	47
3	28/07/06	2.0	26	4	D	0	79	188	114	276	8.78	749	2.73	8.06	14	47
3	28/07/06	2.0	26	4	D	0	68	190	105	265	9.2	741	0.51	8.25	14	47
3	28/07/06	2.0	26	4	D	0	83	189	107	263	9.2	741	0.51	8.25	14	47
3	28/07/06	2.0	26	4	D	0	79	178	104	276	9.2	741	0.51	8.25	14	47
3	28/07/06	3.0	26	4	D	0	82	188	109	285	8.42	740	3.70	8.22	2	6
3	28/07/06	3.0	26	4	D	0	86	190	112	285	8.42	740	3.70	8.22	2	6

3	28/07/06	3.0	26	4	D	0	83	191	110	293	8.42	740	3.70	8.22	2	6
4	31/07/06	1.0	1	4	D	4	76	249	91	249	9.28	951	18.10	7.69		
4	31/07/06	1.0	1	4	D	4	70	233	101	252	9.28	951	18.10	7.69		
4	31/07/06	1.0	1	4	D	4	79	234	98	261	9.28	951	18.10	7.69		
4	31/07/06	2.0	1	4	D	4	70	250	94	257	9.28	951	13.71	7.69		
4	31/07/06	2.0	1	4	D	4	79	269	98	265	9.28	951	13.71	7.69		
4	31/07/06	2.0	1	4	D	4	79	241	97	261	9.28	951	13.71	7.69		
4	31/07/06	3.0	1	4	D	4	68	231	99	261	9.28	951	10.10	7.69		
4	31/07/06	3.0	1	4	D	4	74	249	98	260	9.28	951	10.10	7.69		
4	31/07/06	3.0	1	4	D	4	71	237	102	269	9.28	951	10.10	7.69		
4	31/07/06	1.0	1	11	D	4	76	249	91	249	9.28	951	18.10	7.69		
4	31/07/06	1.0	1	11	D	4	70	233	101	252	9.28	951	18.10	7.69		
4	31/07/06	1.0	1	11	D	4	79	234	98	261	9.28	951	18.10	7.69		
4	31/07/06	2.0	1	11	D	4	70	250	94	257	9.28	951	13.71	7.69		
4	31/07/06	2.0	1	11	D	4	79	269	98	265	9.28	951	13.71	7.69		
4	31/07/06	2.0	1	11	D	4	79	241	97	261	9.28	951	13.71	7.69		
4	31/07/06	3.0	1	11	D	4	68	231	99	261	9.28	951	10.10	7.69		
4	31/07/06	3.0	1	11	D	4	74	249	98	260	9.28	951	10.10	7.69		
4	31/07/06	3.0	1	11	D	4	71	237	102	269	9.28	951	10.10	7.69		
4	31/07/06	1.0	1	20	D	4	76	249	91	249	9.28	951	18.10	7.69		
4	31/07/06	1.0	1	20	D	4	70	233	101	252	9.28	951	18.10	7.69		
4	31/07/06	1.0	1	20	D	4	79	234	98	261	9.28	951	18.10	7.69		
4	31/07/06	2.0	1	20	D	4	70	250	94	257	9.28	951	13.71	7.69		
4	31/07/06	2.0	1	20	D	4	79	269	98	265	9.28	951	13.71	7.69		
4	31/07/06	2.0	1	20	D	4	79	241	97	261	9.28	951	13.71	7.69		
4	31/07/06	3.0	1	20	D	4	68	231	99	261	9.28	951	10.10	7.69		
4	31/07/06	3.0	1	20	D	4	74	249	98	260	9.28	951	10.10	7.69		
4	31/07/06	3.0	1	20	D	4	71	237	102	269	9.28	951	10.10	7.69		
4	01/08/06	1.0	2	20	D	4	71	234	94	258	6.67	948	7.10	7.71		
4	01/08/06	1.0	2	20	D	4	80	240	95	273	6.67	948	7.10	7.71		
4	01/08/06	1.0	2	20	D	4	75	252	97	256	6.67	948	7.10	7.71		
4	01/08/06	1.0	2	20	D	4	78	222	92	241	6.72	946	11.05	7.72		
4	01/08/06	1.0	2	20	D	4	73	225	93	259	6.72	946	11.05	7.72		
4	01/08/06	1.0	2	20	D	4	80	239	92	239	6.72	946	11.05	7.72		
4	01/08/06	2.0	2	20	D	4	80	216	84	243	7.57	910	9.40	7.82		
4	01/08/06	2.0	2	20	D	4	81	222	86	233	7.57	910	9.40	7.82		
4	01/08/06	2.0	2	20	D	4	83	223	87	241	7.57	910	9.40	7.82		
4	01/08/06	3.0	2	20	D	4										
4	01/08/06	3.0	2	20	D	4										
4	01/08/06	3.0	2	20	D	4										
4	01/08/06	1.0	2	11	D	4	79	235	92	239	6.78	930	8.89	7.74		
4	01/08/06	1.0	2	11	D	4	79	230	96	253	6.78	930	8.89	7.74		
4	01/08/06	1.0	2	11	D	4	85	222	93	257	6.78	930	8.89	7.74		
4	01/08/06	1.0	2	11	D	4	80	219	90	243	6.74	931	8.83	7.74		
4	01/08/06	1.0	2	11	D	4	82	228	92	239	6.74	931	8.83	7.74		
4	01/08/06	1.0	2	11	D	4	80	226	88	240	6.74	931	8.83	7.74		
4	01/08/06	2.0	2	11	D	4	81	223	91	239	7.41	934	6.23	7.86		
4	01/08/06	2.0	2	11	D	4	75	228	88	256	7.41	934	6.23	7.86		
4	01/08/06	2.0	2	11	D	4	80	225	92	254	7.41	934	6.23	7.86		
4	01/08/06	3.0	2	11	D	4										
4	01/08/06	3.0	2	11	D	4										
4	01/08/06	3.0	2	11	D	4										
4	01/08/06	1.0	2	4	D	4	78	212	88	246	7.13	925	8.95	7.77		
4	01/08/06	1.0	2	4	D	4	75	219	87	243	7.13	925	8.95	7.77		
4	01/08/06	1.0	2	4	D	4	76	237	93	236	7.13	925	8.95	7.77		
4	01/08/06	1.0	2	4	D	4	77	230	91	249	6.8	924	9.87	7.74		
4	01/08/06	1.0	2	4	D	4	79	232	95	242	6.8	924	9.87	7.74		
4	01/08/06	1.0	2	4	D	4	84	230	90	246	6.8	924	9.87	7.74		
4	01/08/06	2.0	2	4	D	4	75	218	88	241	7.4	926	5.69	7.83		
4	01/08/06	2.0	2	4	D	4	83	217	93	246	7.4	926	5.69	7.83		
4	01/08/06	2.0	2	4	D	4	87	228	93	252	7.4	926	5.69	7.83		
4	01/08/06	3.0	2	4	D	4										
4	01/08/06	3.0	2	4	D	4										
4	01/08/06	3.0	2	4	D	4										
4	02/08/06	1.0	3	20	D	4	77	224	95	240	6.07	933	9.50	7.68	55	26
4	02/08/06	1.0	3	20	D	4	74	228	92	254	6.07	933	9.50	7.68	55	26
4	02/08/06	1.0	3	20	D	4	84	219	94	241	6.07	933	9.50	7.68	55	26
4	02/08/06	1.0	3	20	D	4	80	241	91	248	6.4	916	7.02	7.73	55	26
4	02/08/06	1.0	3	20	D	4	77	224	94	262	6.4	916	7.02	7.73	55	26
4	02/08/06	1.0	3	20	D	4	75	236	95	260	6.4	916	7.02	7.73	55	26
4	02/08/06	2.0	3	20	D	4	75	191	85	231	7.01	922	8.05	7.81	16	10
4	02/08/06	2.0	3	20	D	4	84	219	88	229	7.01	922	8.05	7.81	16	10
4	02/08/06	2.0	3	20	D	4	77	217	88	226	7.01	922	8.05	7.81	16	10
4	02/08/06	3.0	3	20	D	4										
4	02/08/06	3.0	3	20	D	4										

4	02/08/06	3.0	3	20	D	4										
4	02/08/06	1.0	3	11	D	4	80	220	88	228	6.82	918	9.19	7.73		
4	02/08/06	1.0	3	11	D	4	81	220	90	238	6.82	918	9.19	7.73		
4	02/08/06	1.0	3	11	D	4	78	230	100	240	6.82	918	9.19	7.73		
4	02/08/06	1.0	3	11	D	4	78	221	85	233	7.04	924	8.39	7.73		
4	02/08/06	1.0	3	11	D	4	78	226	92	228	7.04	924	8.39	7.73		
4	02/08/06	1.0	3	11	D	4	79	224	92	232	7.04	924	8.39	7.73		
4	02/08/06	2.0	3	11	D	4	72	223	91	231	7.62	928	10.07	7.88		
4	02/08/06	2.0	3	11	D	4	74	208	87	243	7.62	928	10.07	7.88		
4	02/08/06	2.0	3	11	D	4	74	220	89	236	7.62	928	10.07	7.88		
4	02/08/06	3.0	3	11	D	4										
4	02/08/06	3.0	3	11	D	4										
4	02/08/06	3.0	3	11	D	4										
4	02/08/06	1.0	3	4	D	4	74	214	82	227	7.03	924	7.47	7.75		
4	02/08/06	1.0	3	4	D	4	80	226	90	241	7.03	924	7.47	7.75		
4	02/08/06	1.0	3	4	D	4	76	240	90	243	7.03	924	7.47	7.75		
4	02/08/06	1.0	3	4	D	4	75	232	89	255	7.13	928	7.09	7.73		
4	02/08/06	1.0	3	4	D	4	81	227	92	240	7.13	928	7.09	7.73		
4	02/08/06	1.0	3	4	D	4	73	245	91	278	7.13	928	7.09	7.73		
4	02/08/06	2.0	3	4	D	4	71	214	85	233	7.65	920	8.66	7.84		
4	02/08/06	2.0	3	4	D	4	76	210	93	239	7.65	920	8.66	7.84		
4	02/08/06	2.0	3	4	D	4	74	220	88	231	7.65	920	8.66	7.84		
4	02/08/06	3.0	3	4	D	4										
4	02/08/06	3.0	3	4	D	4										
4	02/08/06	3.0	3	4	D	4										
4	03/08/06	1.0	4	20	D	4	88	243	105	269	5.55	931	9.74	7.67	127	149
4	03/08/06	1.0	4	20	D	4	86	236	107	273	5.55	931	9.74	7.67	127	149
4	03/08/06	1.0	4	20	D	4	82	243	106	284	5.55	931	9.74	7.67	127	149
4	03/08/06	1.0	4	20	D	4	78	239	94	256	5.41	918	8.04	7.65	127	149
4	03/08/06	1.0	4	20	D	4	83	231	100	267	5.41	918	8.04	7.65	127	149
4	03/08/06	1.0	4	20	D	4	77	223	92	263	5.41	918	8.04	7.65	127	149
4	03/08/06	2.0	4	20	D	4	73	237	96	278	6.5	928	9.23	7.81	82	78
4	03/08/06	2.0	4	20	D	4	82	219	97	274	6.5	928	9.23	7.81	82	78
4	03/08/06	2.0	4	20	D	4	81	246	96	282	6.5	928	9.23	7.81	82	78
4	03/08/06	3.0	4	20	D	4									4	6
4	03/08/06	3.0	4	20	D	4									4	6
4	03/08/06	3.0	4	20	D	4									4	6
4	03/08/06	1.0	4	11	D	4	84	229	87	238	6.17	922	9.27	7.8		
4	03/08/06	1.0	4	11	D	4	85	213	93	237	6.17	922	9.27	7.8		
4	03/08/06	1.0	4	11	D	4	84	222	91	250	6.17	922	9.27	7.8		
4	03/08/06	1.0	4	11	D	4	80	221	94	250	6.16	925	9.86	7.76		
4	03/08/06	1.0	4	11	D	4	88	225	90	253	6.16	925	9.86	7.76		
4	03/08/06	1.0	4	11	D	4	90	238	94	238	6.16	925	9.86	7.76		
4	03/08/06	2.0	4	11	D	4	84	217	88	240	6.72	931	10.02	7.95		
4	03/08/06	2.0	4	11	D	4	80	230	92	236	6.72	931	10.02	7.95		
4	03/08/06	2.0	4	11	D	4	83	225	94	260	6.72	931	10.02	7.95		
4	03/08/06	3.0	4	11	D	4										
4	03/08/06	3.0	4	11	D	4										
4	03/08/06	3.0	4	11	D	4										
4	03/08/06	1.0	4	4	D	4	72	217	91	251	6.35	924	10.20	7.77		
4	03/08/06	1.0	4	4	D	4	77	234	97	254	6.35	924	10.20	7.77		
4	03/08/06	1.0	4	4	D	4	87	247	92	245	6.35	924	10.20	7.77		
4	03/08/06	1.0	4	4	D	4	84	222	94	234	6.42	920	8.60	7.78		
4	03/08/06	1.0	4	4	D	4	80	239	98	242	6.42	920	8.60	7.78		
4	03/08/06	1.0	4	4	D	4	76	217	97	272	6.42	920	8.60	7.78		
4	03/08/06	2.0	4	4	D	4	78	217	91	245	6.67	923	9.12	7.86		
4	03/08/06	2.0	4	4	D	4	79	232	92	260	6.67	923	9.12	7.86		
4	03/08/06	2.0	4	4	D	4	77	225	97	249	6.67	923	9.12	7.86		
4	03/08/06	3.0	4	4	D	4										
4	03/08/06	3.0	4	4	D	4										
4	03/08/06	3.0	4	4	D	4										
4	04/08/06	1.0	5	20	D	4	81	208	93	256	5.56	918		7.53	199	222
4	04/08/06	1.0	5	20	D	4	73	215	97	247	5.56	918		7.53	199	222
4	04/08/06	1.0	5	20	D	4	87	221	93	243	5.56	918		7.53	199	222
4	04/08/06	1.0	5	20	D	4	81	218	96	246	5.7	921		7.61	199	222
4	04/08/06	1.0	5	20	D	4	80	219	94	240	5.7	921		7.61	199	222
4	04/08/06	1.0	5	20	D	4	76	225	93	250	5.7	921		7.61	199	222
4	04/08/06	2.0	5	20	D	4	85	219	94	247	6.89	919		7.89	301	275
4	04/08/06	2.0	5	20	D	4	82	217	95	243	6.89	919		7.89	301	275
4	04/08/06	2.0	5	20	D	4	83	229	100	251	6.89	919		7.89	301	275
4	04/08/06	3.0	5	20	D	4	76	224	88	238	6.61	921		8.32	5	6
4	04/08/06	3.0	5	20	D	4	71	228	91	246	6.61	921		8.32	5	6
4	04/08/06	3.0	5	20	D	4	75	219	91	254	6.61	921		8.32	5	6
4	04/08/06	1.0	5	11	D	4	79	219	93	247	6.51	921		7.76	25	17
4	04/08/06	1.0	5	11	D	4	81	236	91	238	6.51	921		7.76	25	

4	04/08/06	1.0	5	11	D	4	84	236	97	248	6.51	921		7.76	25	17
4	04/08/06	1.0	5	11	D	4	74	226	91	246	6.57	916		7.63	25	17
4	04/08/06	1.0	5	11	D	4	82	227	96	245	6.57	916		7.63	25	17
4	04/08/06	1.0	5	11	D	4	81	228	89	241	6.57	916		7.63	25	17
4	04/08/06	2.0	5	11	D	4	80	231	95	255	7.12	917		7.89		
4	04/08/06	2.0	5	11	D	4	78	241	93	247	7.12	917		7.89		
4	04/08/06	2.0	5	11	D	4	81	224	97	254	7.12	917		7.89		
4	04/08/06	3.0	5	11	D	4	77	216	89	251	6.27	921		8.58		
4	04/08/06	3.0	5	11	D	4	76	229	93	245	6.27	921		8.58		
4	04/08/06	3.0	5	11	D	4	78	221	92	257	6.27	921		8.58		
4	04/08/06	1.0	5	4	D	4	75	222	93	242	6.68	917		7.76		
4	04/08/06	1.0	5	4	D	4	76	234	93	237	6.68	917		7.76		
4	04/08/06	1.0	5	4	D	4	82	213	90	244	6.68	917		7.76		
4	04/08/06	1.0	5	4	D	4	77	215	87	245	6.89	911		7.77		
4	04/08/06	1.0	5	4	D	4	72	225	93	240	6.89	911		7.77		
4	04/08/06	1.0	5	4	D	4	81	221	93	249	6.89	911		7.77		
4	04/08/06	2.0	5	4	D	4	79	215	94	244	7.12	930		7.88		
4	04/08/06	2.0	5	4	D	4	86	229	95	246	7.12	930		7.88		
4	04/08/06	2.0	5	4	D	4	88	233	94	251	7.12	930		7.88		
4	04/08/06	3.0	5	4	D	4	80	207	89	243	6.74	916		8.4		
4	04/08/06	3.0	5	4	D	4	76	223	89	232	6.74	916		8.4		
4	04/08/06	3.0	5	4	D	4	77	228	90	245	6.74	916		8.4		
4	11/08/06	1.0	12	20	D	4	75	204	89	216	6.24	925	7.11	7.42	225	233
4	11/08/06	1.0	12	20	D	4	80	197	88	238	6.24	925	7.11	7.42	225	233
4	11/08/06	1.0	12	20	D	4	73	213	90	239	6.24	925	7.11	7.42	225	233
4	11/08/06	1.0	12	20	D	4	71	211	94	246	6.15	929	7.04	7.41	225	233
4	11/08/06	1.0	12	20	D	4	73	215	91	241	6.15	929	7.04	7.41	225	233
4	11/08/06	1.0	12	20	D	4	76	200	91	239	6.15	929	7.04	7.41	225	233
4	11/08/06	2.0	12	20	D	4	75	188	92	233	6.74	923	7.30	7.56	301	316
4	11/08/06	2.0	12	20	D	4	78	201	87	227	6.74	923	7.30	7.56	301	316
4	11/08/06	2.0	12	20	D	4	76	216	92	237	6.74	923	7.30	7.56	301	316
4	11/08/06	3.0	12	20	D	4	74	207	88	242	7.51	931	6.41	8.14	175	46
4	11/08/06	3.0	12	20	D	4	71	198	86	251	7.51	931	6.41	8.14	175	46
4	11/08/06	3.0	12	20	D	4	78	207	90	243	7.51	931	6.41	8.14	175	46
4	11/08/06	1.0	12	11	D	4	72	220	88	229	6.7	922	9.38	7.6	125	210
4	11/08/06	1.0	12	11	D	4	71	207	89	252	6.7	922	9.38	7.6	125	210
4	11/08/06	1.0	12	11	D	4	69	202	91	240	6.7	922	9.38	7.6	125	210
4	11/08/06	1.0	12	11	D	4	73	213	87	258	6.6	911	9.61	7.57	125	210
4	11/08/06	1.0	12	11	D	4	78	211	89	247	6.6	911	9.61	7.57	125	210
4	11/08/06	1.0	12	11	D	4	75	207	92	238	6.6	911	9.61	7.57	125	210
4	11/08/06	2.0	12	11	D	4	78	216	92	244	7.71	924	9.97	7.8	189	244
4	11/08/06	2.0	12	11	D	4	75	233	94	249	7.71	924	9.97	7.8	189	244
4	11/08/06	2.0	12	11	D	4	77	226	96	241	7.71	924	9.97	7.8	189	244
4	11/08/06	3.0	12	11	D	4	75	210	93	238	7.84	915	6.76	8.47	9	
4	11/08/06	3.0	12	11	D	4	80	208	93	253	7.84	915	6.76	8.47	9	
4	11/08/06	3.0	12	11	D	4	80	210	91	243	7.84	915	6.76	8.47	9	
4	11/08/06	1.0	12	4	D	4	78	216	93	250	7.72	921	9.42	7.71	26	19
4	11/08/06	1.0	12	4	D	4	87	229	92	254	7.72	921	9.42	7.71	26	19
4	11/08/06	1.0	12	4	D	4	78	227	91	263	7.72	921	9.42	7.71	26	19
4	11/08/06	1.0	12	4	D	4	76	233	91	319	7.6	921	9.19	7.7	26	19
4	11/08/06	1.0	12	4	D	4	75	224	95	251	7.6	921	9.19	7.7	26	19
4	11/08/06	1.0	12	4	D	4	79	213	94	249	7.6	921	9.19	7.7	26	19
4	11/08/06	2.0	12	4	D	4	74	223	92	240	7.9	915	9.43	7.84	8	6
4	11/08/06	2.0	12	4	D	4	82	216	94	260	7.9	915	9.43	7.84	8	6
4	11/08/06	2.0	12	4	D	4	80	216	92	250						
4	11/08/06	3.0	12	4	D	4	82	230	91	235	7.89	917	8.00	8.35	5	
4	11/08/06	3.0	12	4	D	4	79	214	93	238	7.89	917	8.00	8.35	5	
4	11/08/06	3.0	12	4	D	4	79	232	96	247	7.89	917	8.00	8.35	5	
4	18/08/06	1.0	19	20	D	4	67	191	88	219	7.45	914	7.47	7.33	225	233
4	18/08/06	1.0	19	20	D	4	75	172	87	239	7.45	914	7.47	7.33	225	233
4	18/08/06	1.0	19	20	D	4	69	202	86	231	7.45	914	7.47	7.33	225	233
4	18/08/06	1.0	19	20	D	4	73	183	87	216	7.23	914	4.59	7.33	225	233
4	18/08/06	1.0	19	20	D	4	72	178	82	225	7.23	914	4.59	7.33	225	233
4	18/08/06	1.0	19	20	D	4	71	174	87	222	7.23	914	4.59	7.33	225	233
4	18/08/06	2.0	19	20	D	4	75	182	84	229	7.84	915	6.97	7.5	301	316
4	18/08/06	2.0	19	20	D	4	69	200	84	239	7.84	915	6.97	7.5	301	316
4	18/08/06	2.0	19	20	D	4	71	193	88	228	7.84	915	6.97	7.5	301	316
4	18/08/06	3.0	19	20	D	4	73	189	87	236	7.74	936	6.81	7.78	177	155
4	18/08/06	3.0	19	20	D	4	74	216	91	225	7.74	936	6.81	7.78	177	155
4	18/08/06	3.0	19	20	D	4	75	204	90	232	7.74	936	6.81	7.78	177	155
4	18/08/06	1.0	19	11	D	4	70	205	92	243	6.92	917	5.96	7.4	135	254
4	18/08/06	1.0	19	11	D	4	75	198	87	228	6.92	917	5.96	7.4	135	254
4	18/08/06	1.0	19	11	D	4	67	204	90	235	6.92	917	5.96	7.4	135	254
4	18/08/06	1.0	19	11	D	4	71	200	91	235	7.11	908	5.40	7.38	135	254
4	18/08/06	1.0	19	11	D	4	73	204	90	249	7.11	908	5.40	7.38	135	254

4	18/08/06	1.0	19	11	D	4	74	204	91	232	7.11	908	5.40	7.38	135	254
4	18/08/06	2.0	19	11	D	4	77	201	88	234	7.54	914	9.10	7.63	189	285
4	18/08/06	2.0	19	11	D	4	76	211	90	229	7.54	914	9.10	7.63	189	285
4	18/08/06	2.0	19	11	D	4	78	196	92	244	7.54	914	9.10	7.63	189	285
4	18/08/06	3.0	19	11	D	4	68	215	91	251	8.54	920	7.86	8.39	12	
4	18/08/06	3.0	19	11	D	4	79	216	92	254	8.54	920	7.86	8.39	12	
4	18/08/06	3.0	19	11	D	4	81	224	92	245	8.54	920	7.86	8.39	12	
4	18/08/06	1.0	19	4	D	4	79	216	89	241	8.65	910	8.40	7.64	150	70
4	18/08/06	1.0	19	4	D	4	83	214	91	244	8.65	910	8.40	7.64	150	70
4	18/08/06	1.0	19	4	D	4	75	225	89	244	8.65	910	8.40	7.64	150	70
4	18/08/06	1.0	19	4	D	4	74	222	87	241	8.25	908	7.22	7.62	150	70
4	18/08/06	1.0	19	4	D	4	75	225	90	236	8.25	908	7.22	7.62	150	70
4	18/08/06	1.0	19	4	D	4	78	213	91	255	8.25	908	7.22	7.62	150	70
4	18/08/06	2.0	19	4	D	4	74	194	89	232	8.9	913	9.62	7.79	21	32
4	18/08/06	2.0	19	4	D	4	74	220	87	253	8.9	913	9.62	7.79	21	32
4	18/08/06	2.0	19	4	D	4	75	227	90	224	8.9	913	9.62	7.79	21	32
4	18/08/06	3.0	19	4	D	4	72	220	90	232	9.29	920	8.53	8.26	5	1
4	18/08/06	3.0	19	4	D	4	77	217	90	246	9.29	920	8.53	8.26	5	1
4	18/08/06	3.0	19	4	D	4	78	212	91	251	9.29	920	8.53	8.26	5	1
4	25/08/06	1.0	26	20	D	4	68	198	88	224	7.75	925	7.26	7.47	234	240
4	25/08/06	1.0	26	20	D	4	71	187	87	238	7.75	925	7.26	7.47	234	240
4	25/08/06	1.0	26	20	D	4	70	188	85	221	7.75	925	7.26	7.47	234	240
4	25/08/06	1.0	26	20	D	4	69	187	86	222	7.49	925	5.54	7.41	234	240
4	25/08/06	1.0	26	20	D	4	70	175	86	227	7.49	925	5.54	7.41	234	240
4	25/08/06	1.0	26	20	D	4	72	172	86	241	7.49	925	5.54	7.41	234	240
4	25/08/06	2.0	26	20	D	4	73	182	84	220	7.97	926	6.69	7.58	301	316
4	25/08/06	2.0	26	20	D	4	68	191	85	209	7.97	926	6.69	7.58	301	316
4	25/08/06	2.0	26	20	D	4	66	186	84	214	7.97	926	6.69	7.58	301	316
4	25/08/06	3.0	26	20	D	4	71	177	85	254	8.1	930		7.76	177	155
4	25/08/06	3.0	26	20	D	4	67	194	92	241	8.1	930		7.76	177	155
4	25/08/06	3.0	26	20	D	4	79	183	89	246	8.1	930		7.76	177	155
4	25/08/06	1.0	26	11	D	4	78	193	87	219	7.28	912	7.99	7.47	135	254
4	25/08/06	1.0	26	11	D	4	72	188	87	238	7.28	912	7.99	7.47	135	254
4	25/08/06	1.0	26	11	D	4	84	193	92	230	7.28	912	7.99	7.47	135	254
4	25/08/06	1.0	26	11	D	4	79	196	92	223	7.11	924	5.95	7.45	135	254
4	25/08/06	1.0	26	11	D	4	75	203	92	237	7.11	924	5.95	7.45	135	254
4	25/08/06	1.0	26	11	D	4	72	193	94	234	7.11	924	5.95	7.45	135	254
4	25/08/06	2.0	26	11	D	4	73	195	86	226	7.65	907	7.98	7.67	189	285
4	25/08/06	2.0	26	11	D	4	72	207	91	239	7.65	907	7.98	7.67	189	285
4	25/08/06	2.0	26	11	D	4	74	198	93	232	7.65	907	7.98	7.67	189	285
4	25/08/06	3.0	26	11	D	4	79	211	87	226	8.18	914	7.85	8.3	12	1
4	25/08/06	3.0	26	11	D	4	78	206	92	232	8.18	914	7.85	8.3	12	1
4	25/08/06	3.0	26	11	D	4	80	206	92	247	8.18	914	7.85	8.3	12	1
4	25/08/06	1.0	26	4	D	4	75	217	89	229	8.03	915	7.37	7.68	150	120
4	25/08/06	1.0	26	4	D	4	73	212	90	237	8.03	915	7.37	7.68	150	120
4	25/08/06	1.0	26	4	D	4	73	210	92	245	8.03	915	7.37	7.68	150	120
4	25/08/06	1.0	26	4	D	4	79	198	92	243	8.53	914	7.08	7.67	150	120
4	25/08/06	1.0	26	4	D	4	73	204	96	252	8.53	914	7.08	7.67	150	120
4	25/08/06	1.0	26	4	D	4	78	219	97	276	8.53	914	7.08	7.67	150	120
4	25/08/06	2.0	26	4	D	4	76	224	92	245	8.81	930	9.55	7.84	33	73
4	25/08/06	2.0	26	4	D	4	80	222	92	247	8.81	930	9.55	7.84	33	73
4	25/08/06	2.0	26	4	D	4	74	223	94	260	8.81	930	9.55	7.84	33	73
4	25/08/06	3.0	26	4	D	4	82	222	95	247	8.81	917	8.33	8.29	6	2
4	25/08/06	3.0	26	4	D	4	73	226	100	252	8.81	917	8.33	8.29	6	2
4	25/08/06	3.0	26	4	D	4	77	224	97	254	8.81	917	8.33	8.29	6	2
5	25/09/06	1.0	1	4	D	2	84	351	164	431	8.47	537	3.89	7.83		
5	25/09/06	1.0	1	4	D	2	100	318	187	462	8.47	537	3.89	7.83		
5	25/09/06	1.0	1	4	D	2	82	323	167	457	8.47	537	3.89	7.83		
5	25/09/06	2.0	1	4	D	2	90	236	164	424	8.47	537	5.16	7.83		
5	25/09/06	2.0	1	4	D	2	102	247	166	426	8.47	537	5.16	7.83		
5	25/09/06	2.0	1	4	D	2	85	297	164	456	8.47	537	5.16	7.83		
5	25/09/06	3.0	1	4	D	2	74	249	164	424	8.47	537	4.56	7.83		
5	25/09/06	3.0	1	4	D	2	72	316	164	426	8.47	537	4.56	7.83		
5	25/09/06	3.0	1	4	D	2	102	244	164	531	8.47	537	4.56	7.83		
5	25/09/06	1.0	1	11	D	2	84	351	164	431	8.47	537	3.89	7.83		
5	25/09/06	1.0	1	11	D	2	100	318	187	462	8.47	537	3.89	7.83		
5	25/09/06	1.0	1	11	D	2	82	323	167	457	8.47	537	3.89	7.83		
5	25/09/06	2.0	1	11	D	2	90	236	164	424	8.47	537	5.16	7.83		
5	25/09/06	2.0	1	11	D	2	102	247	166	426	8.47	537	5.16	7.83		
5	25/09/06	2.0	1	11	D	2	85	297	164	456	8.47	537	5.16	7.83		
5	25/09/06	3.0	1	11	D	2	74	249	164	424	8.47	537	4.56	7.83		
5	25/09/06	3.0	1	11	D	2	72	316	164	426	8.47	537	4.56	7.83		
5	25/09/06	3.0	1	11	D	2	102	244	164	531	8.47	537	4.56	7.83		
5	25/09/06	1.0	1	20	D	2	84	351	164	431	8.47	537	3.89	7.83		
5	25/09/06	1.0	1	20	D	2	100	318	187	462	8.47	537	3.89	7.83		

5	25/09/06	1.0	1	20	D	2	82	323	167	457	8.47	537	3.89	7.83		
5	25/09/06	2.0	1	20	D	2	90	236	164	424	8.47	537	5.16	7.83		
5	25/09/06	2.0	1	20	D	2	102	247	166	426	8.47	537	5.16	7.83		
5	25/09/06	2.0	1	20	D	2	85	297	164	456	8.47	537	5.16	7.83		
5	25/09/06	3.0	1	20	D	2	74	249	164	424	8.47	537	4.56	7.83		
5	25/09/06	3.0	1	20	D	2	72	316	164	426	8.47	537	4.56	7.83		
5	25/09/06	3.0	1	20	D	2	102	244	164	531	8.47	537	4.56	7.83		
5	26/09/06	1.0	2	20	D	2	88	307	158	412	6.69	526	2.38	7.72		
5	26/09/06	1.0	2	20	D	2	106	347	168	437	6.69	526	2.38	7.72		
5	26/09/06	1.0	2	20	D	2	133	322	163	432	6.69	526	2.38	7.72		
5	26/09/06	2.0	2	20	D	2	88	299	158	412	7.1	521	3.33	7.82		
5	26/09/06	2.0	2	20	D	2	103	339	161	424	7.1	521	3.33	7.82		
5	26/09/06	2.0	2	20	D	2	98	322	159	437	7.1	521	3.33	7.82		
5	26/09/06	2.0	2	20	D	2	78	346	155	394	7.39	532	3.18	7.8		
5	26/09/06	2.0	2	20	D	2	83	326	158	412	7.39	532	3.18	7.8		
5	26/09/06	2.0	2	20	D	2	85	357	150	402	7.39	532	3.18	7.8		
5	26/09/06	3.0	2	20	D	2										
5	26/09/06	3.0	2	20	D	2										
5	26/09/06	3.0	2	20	D	2										
5	26/09/06	1.0	2	11	D	2	96	319	158	405	8.23	5.25	2.06	7.89		
5	26/09/06	1.0	2	11	D	2	91	332	153	435	8.23	5.25	2.06	7.89		
5	26/09/06	1.0	2	11	D	2	80	326	155	429	8.23	5.25	2.06	7.89		
5	26/09/06	2.0	2	11	D	2	95	299	156	420	8.11	530	3.66	7.85		
5	26/09/06	2.0	2	11	D	2	90	301	168	419	8.11	530	3.66	7.85		
5	26/09/06	2.0	2	11	D	2	83	316	159	404	8.11	530	3.66	7.85		
5	26/09/06	2.0	2	11	D	2	85	322	155	425	8.15	532	5.07	7.9		
5	26/09/06	2.0	2	11	D	2	85	321	156	434	8.15	532	5.07	7.9		
5	26/09/06	2.0	2	11	D	2	100	314	158	434	8.15	532	5.07	7.9		
5	26/09/06	3.0	2	11	D	2										
5	26/09/06	3.0	2	11	D	2										
5	26/09/06	3.0	2	11	D	2										
5	26/09/06	1.0	2	4	D	2	88	342	155	410	8.72	530	2.20	7.9		
5	26/09/06	1.0	2	4	D	2	85	339	158	417	8.72	530	2.20	7.9		
5	26/09/06	1.0	2	4	D	2	85	331	156	439	8.72	530	2.20	7.9		
5	26/09/06	2.0	2	4	D	2	96	286	161	420	8.56	531	4.86	8.01		
5	26/09/06	2.0	2	4	D	2	93	314	155	430	8.56	531	4.86	8.01		
5	26/09/06	2.0	2	4	D	2	103	331	159	439	8.56	531	4.86	8.01		
5	26/09/06	2.0	2	4	D	2	71	281	158	415	9.06	526	4.48	7.99		
5	26/09/06	2.0	2	4	D	2	75	281	156	400	9.06	526	4.48	7.99		
5	26/09/06	2.0	2	4	D	2	86	261	156	422	9.06	526	4.48	7.99		
5	26/09/06	3.0	2	4	D	2										
5	26/09/06	3.0	2	4	D	2										
5	26/09/06	3.0	2	4	D	2										
5	27/09/06	1.0	3	20	D	2	79	328	158	421	6.21	531	4.82	7.59	241	150
5	27/09/06	1.0	3	20	D	2	87	320	163	424	6.21	531	4.82	7.59	241	150
5	27/09/06	1.0	3	20	D	2	87	335	160	421	6.21	531	4.82	7.59	241	150
5	27/09/06	2.0	3	20	D	2	109	355	160	470	6.68	532	5.02	7.61		
5	27/09/06	2.0	3	20	D	2	125	389	168	434	6.68	532	5.02	7.61		
5	27/09/06	2.0	3	20	D	2	109	370	163	442	6.68	532	5.02	7.61		
5	27/09/06	2.0	3	20	D	2	89	337	162	419	6.71	533	4.97	7.62		
5	27/09/06	2.0	3	20	D	2	99	333	153	424	6.71	533	4.97	7.62		
5	27/09/06	2.0	3	20	D	2	102	371	165	449	6.71	533	4.97	7.62		
5	27/09/06	3.0	3	20	D	2										
5	27/09/06	3.0	3	20	D	2										
5	27/09/06	3.0	3	20	D	2										
5	27/09/06	1.0	3	11	D	2	87	292	170	424	7.92	529	4.73	7.8		
5	27/09/06	1.0	3	11	D	2	89	282	158	412	7.92	529	4.73	7.8		
5	27/09/06	1.0	3	11	D	2	89	297	158	408	7.92	529	4.73	7.8		
5	27/09/06	2.0	3	11	D	2	87	322	155	411	8.09	528	4.94	7.78		
5	27/09/06	2.0	3	11	D	2	87	342	157	417	8.09	528	4.94	7.78		
5	27/09/06	2.0	3	11	D	2	97	320	153	412	8.09	528	4.94	7.78		
5	27/09/06	2.0	3	11	D	2	84	333	152	411	7.94	527	4.93	7.83		
5	27/09/06	2.0	3	11	D	2	89	350	155	432	7.94	527	4.93	7.83		
5	27/09/06	2.0	3	11	D	2	106	322	155	412	7.94	527	4.93	7.83		
5	27/09/06	3.0	3	11	D	2										
5	27/09/06	3.0	3	11	D	2										
5	27/09/06	3.0	3	11	D	2										
5	27/09/06	1.0	3	4	D	2	89	345	152	401	8.79	529	5.47	7.81		
5	27/09/06	1.0	3	4	D	2	82	323	153	431	8.79	529	5.47	7.81		
5	27/09/06	1.0	3	4	D	2	104	305	162	431	8.79	529	5.47	7.81		
5	27/09/06	2.0	3	4	D	2	92	299	165	447	8.98	531	5.54	7.85		
5	27/09/06	2.0	3	4	D	2	81	304	150	444	8.98	531	5.54	7.85		
5	27/09/06	2.0	3	4	D	2	78	327	160	440	8.98	531	5.54	7.85		
5	27/09/06	2.0	3	4	D	2	104	272	157	412	8.74	530	5.63	7.83		
5	27/09/06	2.0	3	4	D	2	84	277	158	432	8.74	530	5.63	7.83		

5	27/09/06	2.0	3	4	D	2	109	259	160	409	8.74	530	5.63	7.83		
5	27/09/06	3.0	3	4	D	2										
5	27/09/06	3.0	3	4	D	2										
5	27/09/06	3.0	3	4	D	2										
5	28/09/06	1.0	4	20	D	2	79	298	157	394	6.05	519	2.92	7.58	282	202
5	28/09/06	1.0	4	20	D	2	79	310	155	400	6.05	519	2.92	7.58	282	202
5	28/09/06	1.0	4	20	D	2	86	300	161	412	6.05	519	2.92	7.58	282	202
5	28/09/06	2.0	4	20	D	2	110	336	160	414	6.32	529	2.75	7.68	7	
5	28/09/06	2.0	4	20	D	2	104	363	160	415	6.32	529	2.75	7.68	7	
5	28/09/06	2.0	4	20	D	2	124	367	158	419	6.32	529	2.75	7.68	7	
5	28/09/06	2.0	4	20	D	2	87	343	160	402	5.94	531	2.20	7.59	7	
5	28/09/06	2.0	4	20	D	2	96	328	155	420	5.94	531	2.20	7.59	7	
5	28/09/06	2.0	4	20	D	2	92	330	163	424	5.94	531	2.20	7.59	7	
5	28/09/06	3.0	4	20	D	2										
5	28/09/06	3.0	4	20	D	2										
5	28/09/06	3.0	4	20	D	2										
5	28/09/06	1.0	4	11	D	2	84	316	155	409	6.9	531	3.45	7.67	260	61
5	28/09/06	1.0	4	11	D	2	86	333	155	422	6.9	531	3.45	7.67	260	61
5	28/09/06	1.0	4	11	D	2	76	305	161	437	6.9	531	3.45	7.67	260	61
5	28/09/06	2.0	4	11	D	2	99	358	155	405	6.98	529	3.26	7.73		
5	28/09/06	2.0	4	11	D	2	119	356	155	417	6.98	529	3.26	7.73		
5	28/09/06	2.0	4	11	D	2	101	356	161	419	6.98	529	3.26	7.73		
5	28/09/06	2.0	4	11	D	2	102	336	155	409	7.32	530	3.24	7.69		
5	28/09/06	2.0	4	11	D	2	101	344	163	433	7.32	530	3.24	7.69		
5	28/09/06	2.0	4	11	D	2	99	336	157	414	7.32	530	3.24	7.69		
5	28/09/06	3.0	4	11	D	2										
5	28/09/06	3.0	4	11	D	2										
5	28/09/06	3.0	4	11	D	2										
5	28/09/06	1.0	4	4	D	2	87	339	170	394	7.94	529	3.93	7.79		
5	28/09/06	1.0	4	4	D	2	94	338	160	417	7.94	529	3.93	7.79		
5	28/09/06	1.0	4	4	D	2	89	336	163	407	7.94	529	3.93	7.79		
5	28/09/06	2.0	4	4	D	2	97	316	158	455	8.05	531	3.91	7.88		
5	28/09/06	2.0	4	4	D	2	109	320	153	425	8.05	531	3.91	7.88		
5	28/09/06	2.0	4	4	D	2	89	326	155	440	8.05	531	3.91	7.88		
5	28/09/06	2.0	4	4	D	2	99	315	160	407	7.95	536	3.90	7.84		
5	28/09/06	2.0	4	4	D	2	99	336	161	400	7.95	536	3.90	7.84		
5	28/09/06	2.0	4	4	D	2	91	323	157	409	7.95	536	3.90	7.84		
5	28/09/06	3.0	4	4	D	2										
5	28/09/06	3.0	4	4	D	2										
5	28/09/06	3.0	4	4	D	2										
5	29/09/06	1.0	5	20	D	2	93	306	162	412	6.22	524	2.46	7.54	300	300
5	29/09/06	1.0	5	20	D	2	103	310	162	444	6.22	524	2.46	7.54	300	300
5	29/09/06	1.0	5	20	D	2	86	295	167	436	6.22	524	2.46	7.54	300	300
5	29/09/06	2.0	5	20	D	2	88	310	160	439	6.36	527	2.49	7.62	16	5
5	29/09/06	2.0	5	20	D	2	84	308	163	453	6.36	527	2.49	7.62	16	5
5	29/09/06	2.0	5	20	D	2	81	315	162	433	6.36	527	2.49	7.62	16	5
5	29/09/06	2.0	5	20	D	2	76	316	163	453	6.22	532	2.38	7.6	16	5
5	29/09/06	2.0	5	20	D	2	84	328	165	458	6.22	532	2.38	7.6	16	5
5	29/09/06	2.0	5	20	D	2	88	318	165	458	6.22	532	2.38	7.6	16	5
5	29/09/06	3.0	5	20	D	2	103	321	163	444	6.74	525	3.30	7.79	1	5
5	29/09/06	3.0	5	20	D	2	96	389	160	436	6.74	525	3.30	7.79	1	5
5	29/09/06	3.0	5	20	D	2	99	390	162	436	6.74	525	3.30	7.79	1	5
5	29/09/06	1.0	5	11	D	2	74	326	150	429	6.73	522	3.07	7.62	300	169
5	29/09/06	1.0	5	11	D	2	74	268	157	426	6.73	522	3.07	7.62	300	169
5	29/09/06	1.0	5	11	D	2	89	283	155	421	6.73	522	3.07	7.62	300	169
5	29/09/06	2.0	5	11	D	2	91	326	155	429	7.44	524	3.07	7.67	5	
5	29/09/06	2.0	5	11	D	2	104	377	155	426	7.44	524	3.07	7.67	5	
5	29/09/06	2.0	5	11	D	2	96	335	168	433	7.44	524	3.07	7.67	5	
5	29/09/06	2.0	5	11	D	2	84	337	157	412	7.64	530	3.39	7.67	5	
5	29/09/06	2.0	5	11	D	2	103	337	160	433	7.64	530	3.39	7.67	5	
5	29/09/06	2.0	5	11	D	2	101	333	160	453	7.64	530	3.39	7.67	5	
5	29/09/06	3.0	5	11	D	2	99	320	153	407	7.07	528	2.90	8	2	2
5	29/09/06	3.0	5	11	D	2	96	352	163	421	7.07	528	2.90	8	2	2
5	29/09/06	3.0	5	11	D	2	94	325	157	429	7.07	528	2.90	8	2	2
5	29/09/06	1.0	5	4	D	2	86	321	158	417	7.95	530	3.14	7.78		
5	29/09/06	1.0	5	4	D	2	76	325	155	416	7.95	530	3.14	7.78		
5	29/09/06	1.0	5	4	D	2	94	311	168	416	7.95	530	3.14	7.78		
5	29/09/06	2.0	5	4	D	2	89	350	153	412	8.39	526	2.94	7.87		
5	29/09/06	2.0	5	4	D	2	88	318	157	443	8.39	526	2.94	7.87		
5	29/09/06	2.0	5	4	D	2	103	328	160	429	8.39	526	2.94	7.87		
5	29/09/06	2.0	5	4	D	2	98	335	160	421	8.29	531	3.12	7.86		
5	29/09/06	2.0	5	4	D	2	86	330	165	429	8.29	531	3.12	7.86		
5	29/09/06	2.0	5	4	D	2	96	313	167	427	8.29	531	3.12	7.86		
5	29/09/06	3.0	5	4	D	2	99	291	155	412	8.61	533	3.15	8.28		
5	29/09/06	3.0	5	4	D	2	104	296	153	412	8.61	533	3.15	8.28		

5	29/09/06	3.0	5	4	D	2	99	328	155	443	8.61	533	3.15	8.28		
5	06/10/06	1.0	12	20	D	2	77	255	162	444	5.68	537	2.52	7.33	>300	>300
5	06/10/06	1.0	12	20	D	2	77	277	167	456	5.68	537	2.52	7.33	>300	>300
5	06/10/06	1.0	12	20	D	2	72	269	164	426	5.68	537	2.52	7.33	>300	>300
5	06/10/06	2.0	12	20	D	2	90	302	162	426	6.03	539	2.77	7.4	24	10
5	06/10/06	2.0	12	20	D	2	82	329	160	442	6.03	539	2.77	7.4	24	10
5	06/10/06	2.0	12	20	D	2	90	304	164	434	6.03	539	2.77	7.4	24	10
5	06/10/06	2.0	12	20	D	2	75	269	159	429	5.86	534	2.59	7.41	24	10
5	06/10/06	2.0	12	20	D	2	70	269	160	434	5.86	534	2.59	7.41	24	10
5	06/10/06	2.0	12	20	D	2	83	285	159	434	5.86	534	2.59	7.41	24	10
5	06/10/06	3.0	12	20	D	2	97	312	165	432	6.93	537	2.99	7.47	2	16
5	06/10/06	3.0	12	20	D	2	93	336	165	416	6.93	537	2.99	7.47	2	16
5	06/10/06	3.0	12	20	D	2	100	341	162	462	6.93	537	2.99	7.47	2	16
5	06/10/06	1.0	12	11	D	2	98	334	157	441	6.49	534	3.09	7.42	>300	169
5	06/10/06	1.0	12	11	D	2	104	267	157	412	6.49	534	3.09	7.42	>300	169
5	06/10/06	1.0	12	11	D	2	104	324	170	434	6.49	534	3.09	7.42	>300	169
5	06/10/06	2.0	12	11	D	2	72	275	150	421	6.7	531	3.42	7.6	32	18
5	06/10/06	2.0	12	11	D	2	73	287	160	441	6.7	531	3.42	7.6	32	18
5	06/10/06	2.0	12	11	D	2	70	311	154	432	6.7	531	3.42	7.6	32	18
5	06/10/06	2.0	12	11	D	2	88	319	154	444	6.7	526	3.40	7.56	32	18
5	06/10/06	2.0	12	11	D	2	82	326	159	456	6.7	526	3.40	7.56	32	18
5	06/10/06	2.0	12	11	D	2	88	306	164	432	6.7	526	3.40	7.56	32	18
5	06/10/06	3.0	12	11	D	2	95	334	160	426	6.79	534	3.57	7.82	4	13
5	06/10/06	3.0	12	11	D	2	90	329	154	437	6.79	534	3.57	7.82	4	13
5	06/10/06	3.0	12	11	D	2	95	317	162	424	6.79	534	3.57	7.82	4	13
5	06/10/06	1.0	12	4	D	2	80	277	155	421	6.6	532	3.66	7.55	31	17
5	06/10/06	1.0	12	4	D	2	72	302	160	449	6.6	532	3.66	7.55	31	17
5	06/10/06	1.0	12	4	D	2	88	289	162	441	6.6	532	3.66	7.55	31	17
5	06/10/06	2.0	12	4	D	2	93	332	159	427	7.38	531	4.17	7.77	1	
5	06/10/06	2.0	12	4	D	2	97	354	154	416	7.38	531	4.17	7.77	1	
5	06/10/06	2.0	12	4	D	2	92	317	157	429	7.38	531	4.17	7.77	1	
5	06/10/06	2.0	12	4	D	2	92	312	159	432	7.17	532	4.03	7.72	1	
5	06/10/06	2.0	12	4	D	2	97	342	157	436	7.17	532	4.03	7.72	1	
5	06/10/06	2.0	12	4	D	2	95	362	162	444	7.17	532	4.03	7.72	1	
5	06/10/06	3.0	12	4	D	2	85	290	162	411	6.95	532	3.63	7.97		
5	06/10/06	3.0	12	4	D	2	87	317	167	437	6.95	532	3.63	7.97		
5	06/10/06	3.0	12	4	D	2	83	322	167	454	6.95	532	3.63	7.97		
5	13/10/06	1.0	19	20	D	2	89	234	155	412	7.59	538	3.30	7.25	>300	>300
5	13/10/06	1.0	19	20	D	2	87	245	161	433	7.59	538	3.30	7.25	>300	>300
5	13/10/06	1.0	19	20	D	2	82	254	161	428	7.59	538	3.30	7.25	>300	>300
5	13/10/06	2.0	19	20	D	2	79	244	160	430	7.63	536	3.50	7.31	24	14
5	13/10/06	2.0	19	20	D	2	82	272	160	445	7.63	536	3.50	7.31	24	14
5	13/10/06	2.0	19	20	D	2	91	247	158	463	7.63	536	3.50	7.31	24	14
5	13/10/06	2.0	19	20	D	2	77	255	158	402	7.72	532	3.36	7.32	24	14
5	13/10/06	2.0	19	20	D	2	102	265	158	461	7.72	532	3.36	7.32	24	14
5	13/10/06	2.0	19	20	D	2	86	288	160	412	7.72	532	3.36	7.32	24	14
5	13/10/06	3.0	19	20	D	2	81	285	155	414	7.74	530	4.33	7.32	2	16
5	13/10/06	3.0	19	20	D	2	87	288	158	405	7.74	530	4.33	7.32	2	16
5	13/10/06	3.0	19	20	D	2	86	321	158	435	7.74	530	4.33	7.32	2	16
5	13/10/06	1.0	19	11	D	2	92	232	157	407	7.85	536	3.46	7.4	>300	178
5	13/10/06	1.0	19	11	D	2	87	260	157	420	7.85	536	3.46	7.4	>300	178
5	13/10/06	1.0	19	11	D	2	91	262	161	430	7.85	536	3.46	7.4	>300	178
5	13/10/06	2.0	19	11	D	2	104	302	155	427	8.2	535	3.93	7.53	39	20
5	13/10/06	2.0	19	11	D	2	104	290	157	453	8.2	535	3.93	7.53	39	20
5	13/10/06	2.0	19	11	D	2	92	308	161	453	8.2	535	3.93	7.53	39	20
5	13/10/06	2.0	19	11	D	2	87	273	155	460	8.25	530	3.52	7.53	39	20
5	13/10/06	2.0	19	11	D	2	91	247	157	412	8.25	530	3.52	7.53	39	20
5	13/10/06	2.0	19	11	D	2	91	267	153	435	8.25	530	3.52	7.53	39	20
5	13/10/06	3.0	19	11	D	2	105	308	166	430	8.36	530	4.76	7.75	6	15
5	13/10/06	3.0	19	11	D	2	104	318	153	399	8.36	530	4.76	7.75	6	15
5	13/10/06	3.0	19	11	D	2	109	310	161	443	8.36	530	4.76	7.75	6	15
5	13/10/06	1.0	19	4	D	2	92	273	158	437	8.46	534	4.76	7.56	41	49
5	13/10/06	1.0	19	4	D	2	86	297	158	422	8.46	534	4.76	7.56	41	49
5	13/10/06	1.0	19	4	D	2	84	259	155	407	8.46	534	4.76	7.56	41	49
5	13/10/06	2.0	19	4	D	2	89	308	157	432	8.95	530	5.03	7.62	28	2
5	13/10/06	2.0	19	4	D	2	97	308	163	433	8.95	530	5.03	7.62	28	2
5	13/10/06	2.0	19	4	D	2	92	285	153	438	8.95	530	5.03	7.62	28	2
5	13/10/06	2.0	19	4	D	2	107	287	158	415	8.66	534	4.73	7.6	28	2
5	13/10/06	2.0	19	4	D	2	96	285	165	418	8.66	534	4.73	7.6	28	2
5	13/10/06	2.0	19	4	D	2	91	310	161	415	8.66	534	4.73	7.6	28	2
5	13/10/06	3.0	19	4	D	2	112	300	168	414	8.72	532	4.87	7.66		
5	13/10/06	3.0	19	4	D	2	102	315	160	417	8.72	532	4.87	7.66		
5	13/10/06	3.0	19	4	D	2	101	300	168	432	8.72	532	4.87	7.66		
5	20/10/06	1.0	26	20	D	2	79	219	154	420	8.02	540	3.11	7.4	>300	>300
5	20/10/06	1.0	26	20	D	2	97	209	154	416	8.02	540	3.07	7.4	>300	>300

5	20/10/06	1.0	26	20	D	2	76	237	162	418	8.02	540	3.07	7.4	>300	>300
5	20/10/06	2.0	26	20	D	2	71	271	151	382	8.04	539	3.08	7.46	24	14
5	20/10/06	2.0	26	20	D	2	79	245	159	412	8.04	539	3.08	7.46	24	14
5	20/10/06	2.0	26	20	D	2	78	253	160	420	8.04	539	3.08	7.46	24	14
5	20/10/06	2.0	26	20	D	2	71	241	164	428	7.99	540	3.01	7.47	24	14
5	20/10/06	2.0	26	20	D	2	75	235	178	436	7.99	540	3.01	7.47	24	14
5	20/10/06	2.0	26	20	D	2	73	279	160	459	7.99	540	3.01	7.47	24	14
5	20/10/06	3.0	26	20	D	2	78	272	162	392	7.99	536	4.32	7.52	2	16
5	20/10/06	3.0	26	20	D	2	81	279	160	418	7.99	536	4.32	7.52	2	16
5	20/10/06	3.0	26	20	D	2	81	280	156	431	7.99	536	4.32	7.52	2	16
5	20/10/06	1.0	26	11	D	2	66	233	159	413	8.56	539	3.11	7.34	>300	178
5	20/10/06	1.0	26	11	D	2	102	256	156	455	8.56	539	3.11	7.34	>300	178
5	20/10/06	1.0	26	11	D	2	65	237	160	431	8.56	539	3.11	7.34	>300	178
5	20/10/06	2.0	26	11	D	2	68	256	147	400	8.39	545	3.33	7.4	39	22
5	20/10/06	2.0	26	11	D	2	71	259	156	415	8.39	545	3.33	7.4	39	22
5	20/10/06	2.0	26	11	D	2	70	264	159	415	8.39	545	3.33	7.4	39	22
5	20/10/06	2.0	26	11	D	2	79	250	162	412	8.59	540	3.23	7.4	39	22
5	20/10/06	2.0	26	11	D	2	73	258	151	423	8.59	540	3.23	7.4	39	22
5	20/10/06	2.0	26	11	D	2	68	254	162	407	8.59	540	3.23	7.4	39	22
5	20/10/06	3.0	26	11	D	2	86	288	159	402	8.76	540	3.90	7.77	6	15
5	20/10/06	3.0	26	11	D	2	92	324	156	421	8.76	540	3.90	7.77	6	15
5	20/10/06	3.0	26	11	D	2	86	301	156	425	8.76	540	3.90	7.77	6	15
5	20/10/06	1.0	26	4	D	2	79	256	157	434	9.2	540	4.43	7.49	44	50
5	20/10/06	1.0	26	4	D	2	79	300	156	436	9.2	540	4.43	7.49	44	50
5	20/10/06	1.0	26	4	D	2	78	262	157	415	9.2	540	4.43	7.49	44	50
5	20/10/06	2.0	26	4	D	2	78	280	159	395	9.43	542	4.52	7.57	28	9
5	20/10/06	2.0	26	4	D	2	68	269	164	410	9.43	542	4.52	7.57	28	9
5	20/10/06	2.0	26	4	D	2	73	274	164	450	9.43	542	4.52	7.57	28	9
5	20/10/06	2.0	26	4	D	2	78	245	157	421	9.48	542	4.61	7.56	28	9
5	20/10/06	2.0	26	4	D	2	76	266	156	434	9.48	542	4.61	7.56	28	9
5	20/10/06	2.0	26	4	D	2	76	241	156	423	9.48	542	4.61	7.56	28	9
5	20/10/06	3.0	26	4	D	2	89	342	159	434	9.3	543	4.75	7.64		
5	20/10/06	3.0	26	4	D	2	78	297	147	433	9.3	543	4.75	7.64		
5	20/10/06	3.0	26	4	D	2	81	303	159	423	9.3	543	4.75	7.64		
6	13/11/06	3.0	1	4	D	1	3	31	3	8			0.33			
6	13/11/06	3.0	1	4	D	1	3	31	3	8			0.33			
6	13/11/06	3.0	1	4	D	1	3	31	3	8			0.33			
6	13/11/06	3.0	1	4	D	1	3	31	3	8			0.33			
6	13/11/06	1.0	1	4	D	1	2	22	2	7			0.18			
6	13/11/06	1.0	1	4	D	1	2	22	2	7			0.18			
6	13/11/06	1.0	1	4	D	1	2	22	2	7			0.18			
6	13/11/06	1.0	1	4	D	1	2	22	2	7			0.18			
6	13/11/06	2.0	1	4	D	1	1	23	3	5			0.34			
6	13/11/06	2.0	1	4	D	1	1	23	3	5			0.34			
6	13/11/06	2.0	1	4	D	1	1	23	3	5			0.34			
6	13/11/06	2.0	1	4	D	1	1	23	3	5			0.34			
6	13/11/06	3.0	1	11	D	1	3	31	3	8			0.33			
6	13/11/06	3.0	1	11	D	1	3	31	3	8			0.33			
6	13/11/06	3.0	1	11	D	1	3	31	3	8			0.33			
6	13/11/06	3.0	1	11	D	1	3	31	3	8			0.33			
6	13/11/06	1.0	1	11	D	1	2	22	2	7			0.18			
6	13/11/06	1.0	1	11	D	1	2	22	2	7			0.18			
6	13/11/06	1.0	1	11	D	1	2	22	2	7			0.18			
6	13/11/06	1.0	1	11	D	1	2	22	2	7			0.18			
6	13/11/06	2.0	1	11	D	1	1	23	3	5			0.34			
6	13/11/06	2.0	1	11	D	1	1	23	3	5			0.34			
6	13/11/06	2.0	1	11	D	1	1	23	3	5			0.34			
6	13/11/06	2.0	1	11	D	1	1	23	3	5			0.34			
6	13/11/06	3.0	1	20	D	1	3	31	3	8			0.33			
6	13/11/06	3.0	1	20	D	1	3	31	3	8			0.33			
6	13/11/06	3.0	1	20	D	1	3	31	3	8			0.33			
6	13/11/06	3.0	1	20	D	1	3	31	3	8			0.33			
6	13/11/06	1.0	1	20	D	1	2	22	2	7			0.18			
6	13/11/06	1.0	1	20	D	1	2	22	2	7			0.18			
6	13/11/06	1.0	1	20	D	1	2	22	2	7			0.18			
6	13/11/06	1.0	1	20	D	1	2	22	2	7			0.18			
6	13/11/06	2.0	1	20	D	1	1	23	3	5			0.34			
6	13/11/06	2.0	1	20	D	1	1	23	3	5			0.34			
6	13/11/06	2.0	1	20	D	1	1	23	3	5			0.34			
6	13/11/06	2.0	1	20	D	1	1	23	3	5			0.34			
6	14/11/06	3.0	2	4	D	1	2	25	3	32						
6	14/11/06	1.0	2	4	D	1	4	16	2	22						
6	14/11/06	2.0	2	4	D	1	3	34	4	28						
6	14/11/06	3.0	2	20	D	1	3	24	3	37						
6	14/11/06	1.0	2	20	D	1	3	40	4	32						

6	14/11/06	2.0	2	20	D	1	3	26	3	37						
6	14/11/06	3.0	2	11	D	1	2	32	4	36						
6	14/11/06	1.0	2	11	D	1	2	37	3	35						
6	14/11/06	2.0	2	11	D	1	3	48	3	41						
6	16/11/06	3.0	4	4	D	1	3	24	5	47			0.27			
6	16/11/06	1.0	4	4	D	1	2	26	3	45			0.47			
6	16/11/06	2.0	4	4	D	1	3	28	3	42			0.19			
6	16/11/06	3.0	4	20	D	1	4	33	6	65			0.00			
6	16/11/06	1.0	4	20	D	1	5	54	4	66			0.29			
6	16/11/06	2.0	4	20	D	1	3	55	4	54			0.32			
6	16/11/06	3.0	4	11	D	1	3	28	3	45			0.51			
6	16/11/06	1.0	4	11	D	1	3	26	6	74			0.33			
6	16/11/06	2.0	4	11	D	1	2	31	5	40			0.47			
6	17/11/06	3.0	5	4	D	1	3	32	3	38			0.36			
6	17/11/06	1.0	5	4	D	1	2	14	3	41			0.42			
6	17/11/06	2.0	5	4	D	1	3	25	4	31			0.30			
6	17/11/06	3.0	5	20	D	1	3	40	4	87			0.50			
6	17/11/06	3.0	5	20	D	1	3	24	5	86			0.50			
6	17/11/06	1.0	5	20	D	1	3	38	3	33			0.49		3	
6	17/11/06	2.0	5	20	D	1	3	30	7	91			0.55			
6	17/11/06	3.0	5	11	D	1	3	25	4	50			0.36			
6	17/11/06	1.0	5	11	D	1	3	37	4	65			0.36			
6	17/11/06	2.0	5	11	D	1	4	33	4	43			0.41			
6	24/11/06	3.0	12	4	D	1	2	38	3	33			0.36			
6	24/11/06	1.0	12	4	D	1	2	23	3	41			0.30			
6	24/11/06	2.0	12	4	D	1	2	42	4	41			0.04			
6	24/11/06	3.0	12	20	D	1	5	31	8	119			1.01			
6	24/11/06	1.0	12	20	D	1	5	38	5	92			0.80		3	
6	24/11/06	2.0	12	20	D	1	5	46	6	89			1.05			
6	24/11/06	3.0	12	11	D	1	3	35	4	56			0.56			
6	24/11/06	1.0	12	11	D	1	3	36	5	71			0.55			
6	24/11/06	2.0	12	11	D	1	6	41	5	76			0.47			
6	01/12/06	3.0	19	4	D	1	2	16	3	38			0.26			
6	01/12/06	1.0	19	4	D	1	3	35	5	68			0.41			
6	01/12/06	2.0	19	4	D	1	2	22	5	54			0.31			
6	01/12/06	3.0	19	20	D	1	3	49	7	124			0.48			
6	01/12/06	1.0	19	20	D	1	4	40	8	102			1.13		3	
6	01/12/06	2.0	19	20	D	1	5	49	8	98			1.13			
6	01/12/06	3.0	19	11	D	1	3	25	5	101			0.57		1	
6	01/12/06	1.0	19	11	D	1	3	20	5	97			0.59			
6	01/12/06	2.0	19	11	D	1	3	17	5	66			0.68			1
6	08/12/06	3.0	26	4	D	1	3	35	3	60			0.39			
6	08/12/06	1.0	26	4	D	1	3	39	4	59			0.36			
6	08/12/06	2.0	26	4	D	1	5	25	3	35			0.39			
6	08/12/06	3.0	26	20	D	1	4	59	13	117			1.51		1	
6	08/12/06	1.0	26	20	D	1	6	37	10	169			1.36		3	
6	08/12/06	2.0	26	20	D	1	6	39	13	174			1.59			
6	08/12/06	3.0	26	11	D	1	4	22	4	93			0.93		1	
6	08/12/06	1.0	26	11	D	1	3	31	5	114			0.92			
6	08/12/06	2.0	26	11	D	1	4	26	5	83			0.84			1

APPENDIX 5

Date	Cycle	Sample Name	Peak T1			Peak T2			Peak C			Peak A		
			Ex	Em	Int	Ex	Em	Int	Ex	Em	Int	Ex	Em	Int
20/11/2006		Tame	283	362	147	232	351	355	331	414	191	239	420	370
22/11/2006	1	Tame	281	368	129	237	372	264	330	414	175	242	414	347
24/11/2006	2	Tame	280	351	97	234	355	249	325	420	158	237	417	306
01/12/2006	3	Tame	283	338	81	231	345	228	323	414	158	237	423	297
08/12/2006	4	Tame	277	334	72	237	367	270	325	412	157	238	411	294
09/01/2007	5	Tame	280	350	71	237	378	254	327	416	143	239	408	267
11/01/2007		Wood	280	371	393	228	362	665	325	419	289	222	413	841
16/01/2007	1	Wood	279	375	348	222	360	585	338	433	260	222	415	782
23/01/2007	2	Wood	277	380	347	222	370	608	337	414	239	223	397	706
25/01/2007	3	Wood	280	374	302	219	365	650	325	426	244	222	408	636
30/01/2007	4	Wood	280	382	291	222	361	522	331	431	224	222	408	599
01/02/2007	5	Wood	283	381	240	225	358	443	321	416	240	228	421	564
20/11/2006		Rea	285	350	69	232	349	224	330	420	146	237	407	319
22/11/2006	1	Rea	285	367	82	234	361	163	325	423	130	236	417	301
24/11/2006	2	Rea	285	371	74	231	359	180	326	417	113	232	411	272
01/12/2006	3	Rea	283	340	43	231	354	164	324	426	104	231	433	239
08/12/2006	4	Rea	285	361	29	230	356	70	325	411	46	232	422	105
09/01/2007	5	Rea	280	350	37	232	342	161	316	419	101	237	411	246
20/11/2006		HBN	282	344	210	233	360	754	316	420	280	237	414	756
22/11/2006	1	HBN	281	354	233	232	368	674	308	416	282	232	406	741
24/11/2006	2	HBN	280	333	130	227	361	564	312	417	208	222	413	574
01/12/2006	3	HBN	280	325	140	231	350	548	312	422	217	232	394	562
08/12/2006	4	HBN	282	359	191	231	342	590	311	422	255	232	422	674
09/01/2007	5	HBN	282	338	157	231	369	674	301	411	268	231	416	653
20/11/2006		HBS	279	339	145	231	369	679	317	413	246	233	403	651
22/11/2006	1	HBS	278	341	137	228	363	564	311	423	229	225	400	606
24/11/2006	2	HBS	285	358	149	232	347	514	305	417	194	231	397	571
01/12/2006	3	HBS	284	333	97	230	344	429	316	412	185	232	398	488
08/12/2006	4	HBS	282	343	134	232	359	538	304	406	223	228	422	585
09/01/2007	5	HBS	280	350	98	231	367	449	321	419	157	232	414	493
20/11/2006		Vale	285	348	135	231	369	505	316	424	168	237	411	470
22/11/2006	1	Vale	283	360	104	231	359	330	306	418	135	232	408	329
24/11/2006	2	Vale	283	349	72	232	352	297	317	418	108	223	416	346
01/12/2006	3	Vale	281	338	63	231	343	272	306	402	118	232	402	295

08/12/2006	4	Vale	282	353	55	232	357	210	304	410	76	231	412	198
09/01/2007	5	Vale	280	350	50	232	347	237	316	422	81	232	421	264
11/01/2007		Bartley	282	344	61	230	342	126	335	421	195	231	425	401
16/01/2007	1	Bartley	284	361	73	233	348	104	345	425	214	235	422	436
23/01/2007	2	Bartley	280	350	48	234	346	128	340	423	197	238	421	432
25/01/2007	3	Bartley	280	350	51	223	342	129	342	424	208	232	421	461
30/01/2007	4	Bartley	280	350	44	223	333	87	327	423	193	233	431	425
01/02/2007	5	Bartley	280	350	40	231	354	148	331	414	195	234	440	431
11/01/2007		Merritt's	282	344	80	233	350	173	341	419	303	236	430	477
16/01/2007	1	Merritt's	282	345	71	232	344	149	338	423	280	236	413	517
23/01/2007	2	Merritt's	280	350	62	232	347	135	331	419	253	237	440	478
25/01/2007	3	Merritt's	280	350	67	232	354	153	330	424	257	237	414	527
30/01/2007	4	Merritt's	280	350	61	231	353	133	336	432	246	234	439	508
01/02/2007	5	Merritt's	280	350	53	234	350	135	335	432	244	234	427	522
11/01/2007		Trent	280	344	96	233	356	229	332	422	322	238	415	544
16/01/2007	1	Trent	280	350	82	232	341	164	342	424	315	232	423	615
23/01/2007	2	Trent	280	350	68	232	345	159	332	429	326	236	426	429
25/01/2007	3	Trent	280	350	70	232	341	143	326	423	287	237	430	590
30/01/2007	4	Trent	280	350	59	230	340	125	332	419	270	237	433	582
01/02/2007	5	Trent	280	350	59	233	358	211	330	425	274	236	419	547
20/11/2006		Repton	280	359	49	233	360	96	321	429	107	238	417	235
22/11/2006	1	Repton	282	370	51	232	353	14	327	407	103	237	426	210
24/11/2006	2	Repton	285	362	38	232	342	37	335	416	93	242	423	177
01/12/2006	3	Repton	283	368	44	232	342	10	331	414	87	237	433	158
08/12/2006	4	Repton	279	366	44	232	335	17	330	412	105	238	415	177
09/01/2007	5	Repton	280	350	24	232	334	21	335	422	96	238	434	196
11/01/2007		Hilton	285	355	133	227	348	316	331	421	486	236	429	852
16/01/2007	1	Hilton	285	372	162	230	348	156	339	427	422	231	436	851
23/01/2007	2	Hilton	280	350	56	232	350	120	329	414	400	232	428	891
25/01/2007	3	Hilton	280	350	70	226	350	124	339	426	417	232	424	870
30/01/2007	4	Hilton	280	350	44	229	356	131	340	431	417	232	424	916
01/02/2007	5	Hilton	280	350	54	229	344	135	343	424	442	236	421	982
20/11/2006		Dove	288	344	38	232	368	143	341	429	165	237	420	317
22/11/2006	1	Dove	285	352	39	234	351	31	337	421	144	237	428	294
24/11/2006	2	Dove	280	366	42	231	350	59	331	420	120	237	413	239
01/12/2006	3	Dove	282	357	31	231	347	11	337	420	112	237	423	209

08/12/2006	4	Dove	284	364	31	231	342	19	335	420	101	233	430	207
09/01/2007	5	Dove	280	350	8	229	350	14	338	430	52	232	422	112
11/01/2007		Alder	278	361	136	228	354	211	340	418	467	237	439	852
16/01/2007	1	Alder	279	354	86	231	336	133	343	428	435	231	420	956
23/01/2007	2	Alder	280	350	56	233	347	120	342	419	431	236	442	866
25/01/2007	3	Alder	280	350	76	229	350	150	332	424	417	230	429	838
30/01/2007	4	Alder	280	350	53	227	351	197	337	420	413	235	436	875
01/02/2007	5	Alder	280	350	77	229	352	148	340	422	407	233	437	943
20/11/2006		18MΩ	280	354	2	231	352	13	336	417	2	227	398	6
22/11/2006	1	18MΩ	285	356	3	212	357	62	319	421	3	222	407	14
24/11/2006	2	18MΩ	279	350	4	214	356	35	323	416	3	223	406	19
01/12/2006	3	18MΩ	281	350	4	212	354	60	322	415	3	220	411	33
08/12/2006	4	18MΩ	284	343	5	218	334	48	325	419	4	223	417	29
09/01/2007	5	18MΩ	281	349	5	224	345	37	318	422	3	239	438	25

APPENDIX 6

Date	Cycle	Sample name	Peak T1			Peak T2			Peak C			Peak A		
			Ex	Em	Int	Ex	Em	Int	Ex	Em	Int	Ex	Em	Int
20/11/2006		Tame	283	362	147	232	351	355	331	414	191	239	420	370
21/11/2006	1	Tame	285	354	103	237	372	293	322	417	162	237	424	326
22/11/2006	2	Tame	285	357	89	235	366	249	316	413	149	238	402	313
24/11/2006	3	Tame	282	364	78	238	373	225	322	423	121	237	399	267
01/12/2006	4	Tame	281	368	79	234	354	150	326	403	123	236	412	250
08/12/2006	5	Tame	282	372	82	232	373	182	336	422	131	236	408	240
11/01/2007		Wood	280	371	396	228	362	689	325	419	291	222	413	857
16/01/2007	1	Wood	282	358	169	223	351	312	338	428	209	223	417	571
23/01/2007	2	Wood	280	350	151	227	362	351	342	425	224	222	420	525
25/01/2007	3	Wood	282	354	121	224	347	271	321	413	257	221	398	646
30/01/2007	4	Wood	287	372	145	230	368	307	320	408	220	224	416	520
01/02/2007	5	Wood	280	350	91	231	361	238	330	427	206	232	422	441
20/11/2006		Rea	285	350	69	232	349	224	330	420	146	237	407	319
21/11/2006	1	Rea	288	356	53	232	361	182	336	418	118	236	419	256
22/11/2006	2	Rea	286	360	59	231	339	84	320	422	112	237	418	252
24/11/2006	3	Rea	284	371	58	234	350	84	321	416	97	235	410	244
01/12/2006	4	Rea	282	343	28	228	350	52	329	410	91	232	411	218
08/12/2006	5	Rea	285	362	46	231	350	84	341	424	88	231	410	201
20/11/2006		HBN	282	344	210	233	360	754	316	420	280	237	414	756
21/11/2006	1	HBN	285	362	169	232	365	534	316	415	199	232	400	527
22/11/2006	2	HBN	282	366	174	232	371	535	304	423	211	232	419	529
24/11/2006	3	HBN	283	362	148	230	352	420	306	420	216	231	420	558
01/12/2006	4	HBN	282	369	120	227	365	317	315	431	161	230	421	408
08/12/2006	5	HBN	283	370	204	233	369	552	306	430	280	232	417	703
20/11/2006		HBS	281	342	134	231	345	534	300	416	220	231	407	554
21/11/2006	1	HBS	281	366	150	232	376	468	306	412	182	231	410	477
22/11/2006	2	HBS	282	334	76	232	358	403	301	428	172	232	400	416
24/11/2006	3	HBS	282	344	70	231	355	283	305	415	151	232	402	379
01/12/2006	4	HBS	286	359	83	232	371	254	306	411	138	231	432	349
08/12/2006	5	HBS	282	366	45	233	361	132	309	409	72	232	417	181
20/11/2006		Vale	285	348	135	231	369	505	316	424	168	237	411	470
21/11/2006	1	Vale				232	364	395	306	412	145	231	408	373
22/11/2006	2	Vale	284	360	97	232	364	322	312	423	131	232	420	361
24/11/2006	3	Vale	283	364	90	232	356	269	305	407	118	233	415	322

01/12/2006	4	Vale	292	374	110	232	366	238	303	418	121	232	411	319
08/12/2006	5	Vale	283	360	68	231	368	243	304	423	104	227	414	286
11/01/2007		Bartley	282	344	64	230	342	149	335	421	197	231	425	417
16/01/2007	1	Bartley	280	350	47	232	340	66	340	426	201	233	416	420
23/01/2007	2	Bartley	280	350	26	233	347	47	341	428	179	234	433	367
25/01/2007	3	Bartley	280	350	26	231	341	40	311	429	143	231	424	346
30/01/2007	4	Bartley	280	350	26	232	354	64	342	422	142	235	442	348
01/02/2007	5	Bartley	280	350	32	232	348	59	341	421	140	229	411	313
11/01/2007		Merritt's	282	344	83	233	350	197	341	419	305	236	430	493
16/01/2007	1	Merritt's	280	350	48	232	349	111	332	436	217	237	420	422
23/01/2007	2	Merritt's	280	350	42	232	340	26	334	420	207	236	434	416
25/01/2007	3	Merritt's	280	350	41	232	339	57	331	423	241	232	435	457
30/01/2007	4	Merritt's	280	350	41	222	353	108	341	428	215	234	427	431
01/02/2007	5	Merritt's	280	350	41	231	340	51	329	423	208	239	431	387
11/01/2007		Trent	280	344	99	233	356	252	332	422	324	238	415	560
16/01/2007	1	Trent	281	353	58	234	344	118	333	432	257	233	436	516
23/01/2007	2	Trent	280	350	54	232	341	70	334	416	267	235	429	494
25/01/2007	3	Trent	280	35	52	230	333	65	317	417	247	236	426	515
30/01/2007	4	Trent	280	350	36	233	341	86	335	420	253	235	427	507
01/02/2007	5	Trent	280	350	47	232	342	93	330	427	251	236	423	473
20/11/2006		Repton	280	359	49	233	360	96	321	429	107	238	417	235
21/11/2006	1	Repton	283	376	64	237	346	46	333	423	90	237	421	173
22/11/2006	2	Repton	285	354	28	233	346	8	340	424	93	241	428	159
24/11/2006	3	Repton	280	374	37	232	350	8	336	424	80	237	413	154
01/12/2006	4	Repton	281	363	24	232	352	-17	341	430	74	236	434	146
08/12/2006	5	Repton	284	371	33	233	358	37	335	426	76	236	419	130
11/01/2007		Hilton	285	355	136	227	348	340	331	421	488	236	429	868
16/01/2007	1	Hilton	280	350	54	234	349	138	326	421	404	238	433	917
23/01/2007	2	Hilton	280	350	51	234	354	89	325	429	345	231	431	728
25/01/2007	3	Hilton	280	350	41	232	350	97	317	427	379	232	423	786
30/01/2007	4	Hilton	280	350	42	223	352	220	331	421	338	234	419	739
01/02/2007	5	Hilton	280	350	45	229	358	143	341	428	327	234	423	668
20/11/2006		Dove	288	344	38	232	368	143	341	429	165	237	420	317
21/11/2006	1	Dove	286	355	35	231	337	47	333	425	129	230	433	250
22/11/2006	2	Dove	284	366	44	232	343	25	343	430	134	237	412	253

24/11/2006	3	Dove	285	368	44	232	354	41	341	430	114	237	424	241
01/12/2006	4	Dove	285	364	37	233	346	-8	331	424	110	238	423	216
08/12/2006	5	Dove	279	368	38	231	342	31	335	421	98	232	431	202
11/01/2007		Alder	278	361	139	228	354	235	340	418	469	237	439	868
16/01/2007	1	Alder	282	353	83	231	358	164	321	427	397	232	417	799
23/01/2007	2	Alder	280	350	48	228	352	89	336	429	351	232	421	709
25/01/2007	3	Alder	280	350	57	223	351	149	321	428	375	233	417	757
30/01/2007	4	Alder	280	350	58	227	348	116	336	419	351	232	429	736
01/02/2007	5	Alder	280	350	42	225	350	124	340	424	330	238	439	703
11/01/2007		18MΩ distilled	282	354	1	223	350	19	330	418	1	238	443	5
16/01/2007	1	18MΩ distilled	280	350	3	219	368	31	321	419	5	223	404	22
23/01/2007	2	18MΩ distilled	280	350	2	216	361	61	320	422	5	218	404	34
25/01/2007	3	18MΩ distilled	282	352	4	214	366	50	322	416	6	228	411	19
30/01/2007	4	18MΩ distilled	280	350	2	218	361	32	320	420	6	216	406	31
01/02/2007	5	18MΩ distilled	280	350	3	217	367	44	320	420	5	219	404	61

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