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UV-visible absorbance spectroscopy as a proxy for peatland dissolved organic carbon (DOC) quantity and quality: considerations on wavelength and absorbance degradation

Mike Peacock[ac], Chris D. Evans[b], Nathalie Fenner[a], Chris Freeman[a], Rachel Gough[a], Timothy G. Jones[a], Inma Lebron[b].

[a]Wolfson Carbon Capture Laboratory, School of Biological Sciences, Bangor University, Deiniol Road, LL57 2UW, UK.

[b]Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Road, LL57 2UW, UK.

[c]*Present address*: Centre for Earth, Planetary, Space and Astronomical Research (CEPSAR), Department of Environment, Earth and Ecosystems, The Open University, Walton Hall, Milton Keynes, MK7 6AA. 01908655129

Abstract

Absorbance in the UV or visible spectrum (UV-Vis) is commonly used as a proxy for DOC concentrations in waters draining upland catchments. To determine the appropriateness of different UV-Vis measurements we used surface and pore water samples from two Welsh peatlands in four different experiments:

i) An assessment of single wavelength proxies (1 nm increments between 230-800 nm) for DOC concentration demonstrated that 254 nm was more accurate than 400 nm. The highest R^2 values between absorbance and DOC concentration were generated using 263 nm for one sample set ($R^2 = 0.91$), and 230 nm for the other three sample sets (respective R^2 values of 0.86, 0.81, and 0.93).

ii) A comparison of different DOC concentration proxies, including single wavelength proxies, a two wavelength model, a proxy using phenolic concentration, and a proxy using the area under a UV spectrum at 250-350 nm. It was found that both a single wavelength proxy (≤ 263 nm) and a two wavelength model performed well for both pore water and surface water.

iii) An evaluation of the E2:E3, E2:E4, E4:E6 ratios, and SUVA (absorbance at 254 nm normalised to DOC concentration) as indicators of DOC quality showed that the E4:E6 ratio was subject to extensive variation over time, and was highly correlated between surface water and pore water, suggesting that it is a useful metric to determine temporal changes in DOC quality.

iv) A repeated weekly analysis over twelve weeks showed no consistent change in UV-Vis absorbance, and therefore an inferred lack of degradation of total DOC in samples that were filtered and stored in the dark at 4°C.

Keywords: Peatland, dissolved organic carbon, UV-visible spectroscopy, absorbance, water colour, E4:E6 ratio,

1. Introduction

Dissolved organic carbon (DOC) is a ubiquitous component of the freshwater carbon cycle. It attenuates UV radiation[1], influences the functioning of aquatic ecosystems[2], impacts on water treatment costs[3], and has implications for human health in potable water due to its role as a precursor of disinfectant by-products[4]. Large increases in DOC concentrations have been observed in surface waters draining semi-natural ecosystems across many areas of

Northern Europe and Northeastern North America during the last 20-40 years[5,6,7]. While these increases have been widely attributed to ecosystem recovery from acid deposition[7,8,9], many other alternative or additional drivers have also been proposed[5,10,11,12]. Given this continued uncertainty, the magnitude of observed change, and the ecological, economic and potential health consequences of DOC, the analysis of DOC concentrations in natural waters has become increasingly widespread.

The controls on production and consumption of DOC are somewhat complex, and include factors such as vegetation composition, hydrology and soil chemistry. For instance, vegetation can influence local hydrology and soil temperature, and plants can stimulate microbial activity through the release of root exudates. These will affect rates of decomposition and therefore DOC concentrations[13]. It has been demonstrated that soil acidity directly affects DOC concentrations[8], because a higher pH increases the solubilisation of DOC[14]. Furthermore, pore water DOC concentrations can vary across small spatial scales, and it has been suggested that hotspots of decomposition are driven by elevated activities of extracellular enzymes. These high enzyme activities are associated with the presence of electron acceptors such as iron, the availability of labile nutrients, and pH changes[15]. Finally, there are simple hydrological mechanisms that control DOC concentrations; a greater flow of water through a soil profile will flush out more DOC, and the magnitude of this will depend on how often this flushing occurs[16].

DOC is commonly measured directly using two laboratory methods. DOC can be measured as ‘non-purgeable’ organic carbon (NPOC) by converting inorganic carbon to carbon dioxide (CO_2) and purging this CO_2. The remaining organic carbon is then converted to CO_2 by thermal oxidation and measured by absorbance of infra-red radiation. The second method involves measuring total carbon (TC) and inorganic carbon (IC) and deriving DOC by subtracting IC from TC. Both of these methods require access to relatively expensive analytical equipment. Other, cheaper options exist, such as the method that uses a chemically-induced colour change to measure DOC[17]. A simpler method is to use UV-visible (UV-Vis) spectroscopy, as the absorbance of light by water from natural systems is highly dependent on DOC concentration, therefore absorbance can be used as a proxy for DOC concentration[18]. In the water treatment industry, absorbance at 254 nm is often used as a surrogate for DOC because aromatic humic substances are the dominant component of DOC in natural waters, and these absorb light in the UV (and to a lesser extent visible) part of the electromagnetic spectrum[19]. Humic substances can comprise up to 90% of DOC in some lakes and wetlands, although their contribution can vary considerably[20]. The link between aromaticity and absorbance at 254 nm has been demonstrated directly using ^{13}C NMR spectroscopy[21]. By establishing a calibration between a number of paired DOC and absorbance values, DOC concentrations can be calculated relatively cheaply and quickly by just measuring absorbance.

Apart from 254 nm, numerous other wavelengths have been used as proxies for DOC concentration, listed in Table 1. Occasionally DOC is calculated for a specific site using a calibration generated elsewhere[37], but Wallage & Holden[33] caution against this, as calibrations can vary according to factors such as soil type, vegetation, peat depth and land management, as well as with time due to variations in DOC structural characteristics. However, the study in question[33] investigated 400 nm as a proxy which is within the visible spectrum. Absorbance in visible wavelengths is used in the water industry as it is important to the aesthetic quality of treated waters, but as humic substances are coloured to varying degrees[20] a wavelength in this region may not be the most appropriate proxy for total DOC. Measuring absorbance at a lower wavelength in the UV spectrum may produce more robust calibrations[38].

More sophisticated methods to calculate DOC concentration using light absorbance have been proposed, such as the use of two wavelengths[39,40], multiple wavelengths[41], and the method of Wang & Hsieh[38] which uses the area under the UV-Vis spectra as a proxy. Chemical methods of estimating DOC also exist, such as that proposed by Peacock *et al.*[42] which uses the concentration of phenolics in a water sample as a DOC proxy. Phenolics are plant metabolites and a type of DOC[43]. Despite the availability of these methods, numerous environmental studies continue to rely on calibrations using one wavelength[30,44]. In light of this, a thorough investigation of the appropriateness of different wavelength proxies is needed, as well as a comparison of different methods.

In addition to being used as a proxy for DOC concentration, UV-Vis spectroscopy is also used as a tool to provide information on the structure and composition of DOC (table 2). The ‘E4:E6’ ratio is frequently cited as a measure of humification or molecular weight[20,53], and is the ratio of absorbance at two wavelengths; one in the region of 400 nm and one in the region of 600 nm. Similarly, the ‘E2:E3’ ratio (absorbance at 250 nm and 365 nm) is used as an estimation of aromaticity and molecular weight[45]. ‘SUVA’ (specific UV absorbance: an absorbance measurement, usually taken at 254 nm, divided by DOC concentration) is also a measure of aromaticity[21] and has been positively correlated with DOC hydrophobicity and molecular weight[54]. E2:E4 ratios are sometimes used, where absorbance is measured at two wavelengths; one around 200 nm and one around 400 nm. This ratio has been cited as a measure of humification[48], and as a comparison of the UV-absorbing functional groups and coloured ones in DOC[46,47]. Similarly, spectral slope ratios can provide information on molecular weight[55]. However, doubt has been expressed over the use and applicability of some of these ratios; for instance O’Driscoll *et al.*[56] observed no correlation between the E4:E6 ratio and other measures of DOC character.

In view of the prevalence of spectrophotometric analysis, it is also worth considering how long a water sample can be retained in storage before analysis, and still produce an accurate and reliable result. Current practice is to measure absorbance as soon as possible after sampling, often within one day (e.g. Wilson *et al.*[44]) so as to limit the effects of biological or physicochemical degradation. Under the Disinfectant/Disinfection By-products Rule of the US Environmental Protection Agency, samples for UV scanning must be analysed within two days[57]. However, there does not appear to be any detailed study in the literature describing the changes in absorbance of stored samples over time.

The aim of this experiment is therefore four-fold:

1. To investigate the appropriateness of different wavelengths as proxies for DOC concentration, and to observe whether this changes for different sets of samples (hereafter referred to as the "DOC single wavelength proxy assessment").
2. To compare different indirect methods of DOC measurement (a two wavelength model, a phenolics proxy, the area under an absorbance spectrum, and a simple calibration using a single wavelength) (hereafter referred to as the "alternative DOC proxy comparison").
3. To assess the suitability of the E2:E3, E2:E4, and E4:E6 ratios, and SUVA (hereafter referred to as the "E ratio and SUVA assessment").
4. To repeatedly measure the weekly change (if any) in absorbance for a set of water samples (hereafter referred to as the "absorbance degradation" experiment).

Taken together, the findings will enable recommendations to be made regarding the use of UV-Vis spectroscopy as a tool to analyse DOC concentration and quality in natural waters.

2. Materials and Methods

2.1. Site descriptions and sampling

Samples were taken from two ombrotrophic peatlands in north Wales, UK. The catchment of the Afon Ddu (latitude 52.97°N, longitude 3.84°W) is part of the Migneint blanket bog. Dominant vegetation is *Calluna vulgaris* with some *Eriophorum* and *Sphagnum* species. Mean annual air temperature is 8.6^{o}C and mean annual precipitation is 2200 mm[58]. The altitude is 490 m above sea level. Three sets of samples were from an experimental site within which recent peat restoration has been undertaken by blocking old drainage ditches, within a replicated and controlled experimental design (note that the restoration work did not appear to have any immediate effects on DOC concentration or quality). Samples were collected from i): surface water from twelve open or blocked ditches, ii) pore water samples taken from twelve piezometers at 10 cm depth, and iii) overland-flow surface water samples collected from twenty four crest-stage tubes. Samples were collected between January 2011 and October 2012.

The catchment of the Alwen Reservoir (latitude 53.07°N, longitude 3.57°W) is 21 km from the Afon Ddu catchment. The catchment comprises some blanket peat dominated by *Sphagnum* species, alongside *Calluna vulgaris* and *Eriophorum*. Approximately 30% of the catchment is afforested, the main species being *Picea sitchensis* and *Picea abies*, plus some *Larix kaempferi, Fagus sylvatica* and *Pinus sylvestris*. The altitude is 370 m above sea level. Mean annual air temperature is 8.6^{o}C and mean annual precipitation is 1430 mm[59]. Samples from the Alwen Reservoir catchment were taken monthly from six streams from October 2011 to May 2012. The soils of the sub-catchments of the six streams were either predominantly peat or acid organo-mineral upland soils. The habitats varied and included unimproved acid grassland, dry acid heath, blanket bog, forest plantation, and acid/neutral flush.

After collection, water samples were stored in the dark at 4^{o}C before analysis and filtration. Analysis always took place within one week of collection. Samples were filtered through Whatman 0.45 µm cellulose nitrate filters and analysed for DOC using an Analytical Sciences Ltd Thermalox Total Carbon analyser, equipped with a CO_2 detector. Samples were acidified (pH < 3) with 1M hydrochloric acid, sparged with oxygen to remove any inorganic carbon, and DOC concentrations calculated using a seven point calibration curve (potassium hydrogen phthalate, concentrations: 5, 10, 20, 30, 40, 50, 60 mg L^{-1}), with additional standards (selected to be close to the expected concentration of the samples) to check for drift, (plus a quality control sample of 20 mg L^{-1} D-glucose in ultrapure water). Several samples (1-3 per run) were duplicated to check for reproducibility. Each individual sample was injected 5 times, and the result accepted if the coefficient of variation of the five injections was less than 3%. If drift was detected (deviation from the real value of more than 5%), the last set of standards at the end of the batch were used to correct for the drift by using linear regression and recalculating the DOC values from the point where the drift was detected.

UV-Vis analysis was conducted using a Molecular Devices M2e Spectramax plate-reader (wavelength accuracy ± 2 nm, photometric accuracy ± 0.006, photometric precision ± 0.003). 348 µl of sample was pipetted (pipettes calibrated externally by Starlab on an annual basis) onto a microplate and wavelengths were scanned on a 1 nm increment from 230 nm to 800 nm. Results were corrected against blanks of ultrapure water (three blanks per 96-well plate) and converted to values equivalent to those obtained using the standard cuvette method. This was done using multiplication factors that were generated by comparing cuvette and microplate analysis of 29 samples from contrasting locations (upland soil water, upland acidic freshwater, lowland alkaline freshwater). Absorbance of the samples ranged from 0.05 to 3.83 at 254 nm. For each wavelength, a linear regression was fitted between the cuvette and microplate results for a range of 254 nm absorbance categories (<0.1, 0.1-0.5, >0.5) and the slope value used as the multiplication factor[60].

Phenolic concentrations were determined using a method adapted from Box[61]. 0.25 ml of sample was added to a clear microplate well. 12.5 μl of Folin-Ciocalteau reagent was added followed by 37.5 μl of Na_2CO_3 (200 g L^{-1}). After 1.5 hours the absorbance was measured at 750nm on a Molecular Devices M2e Spectramax plate-reader. Phenolic concentrations were then derived from the preparation of a standard curve using laboratory-prepared phenol standards of known concentration (0, 1, 2, 4, 6, 8, 10, 15, 20 mg L^{-1}).

Data analysis was performed using SPSS v16.0.1 (IBM Corporation).

2.2. DOC single wavelength proxy assessment

For each set of samples a regression was performed between each individual wavelength (between 230 nm and 800 nm) and DOC concentration, with the aim of determining which wavelength gave the highest R^2 value. For the Afon Ddu catchment, monthly ditch and piezometer samples from July 2011 to January 2012 were included in the analysis, and overland-flow samples from January and July 2012 were used. For the Alwen Reservoir catchment all data was used (from October 2011 to May 2012).

2.3. Alternative DOC proxy comparison

In order to compare different procedures for indirect DOC estimations, we selected six different methods from the literature. These were:

1. A two wavelength model proposed by Tipping *et al.*[39] and advanced by Carter *et al.*[40]. This model calculates DOC using absorbance at 270 nm and 350 nm alone, and was created using measurements of DOC and UV-vis from a variety of surface water samples.

2. The method of Wang & Hsieh[38], that uses the area under the UV spectra between 250 nm and 350 nm to create a calibration for DOC.
3. A calibration curve created using absorbance at 254 nm (refer to table 1).
4. A calibration curve created using absorbance at 400 nm (table 1).
5. A calibration created using the optimum absorbance wavelength derived from the DOC single wavelength proxy assessment.
6. The method of Peacock *et al.*[42], that uses a calibration created using phenolic concentration.

All six methods were tested on two sets of samples from the Afon Ddu catchment; one set from ditch water, and one set from pore water; and a set of stream samples from the catchment of the Alwen Reservoir. Different sets of samples were used for both the creation and testing of the models. Details of which samples were collected and used for the model creation and testing are in table 3.

To test the different models several metrics were used. These were:

1. The mean absolute difference (in mg L^{-1}) between modelled and measured DOC concentrations.
2. The Nash-Sutcliffe model efficiency coefficient[62], calculated as:

$$NS = 1 - \frac{\Sigma\,(DOC\ meas - DOC\ mod)^2}{\Sigma\,(DOC\ meas - DOC\ mean)^2}$$

where ‘DOC meas’ is the actual measured concentration of DOC, ‘DOC mod’ is the concentration of DOC modelled using the various proxies, and ‘DOC mean’ is the mean concentration of the actual DOC measurements. A NS value of 1 indicates a perfect model fit, and as the NS value decreases this indicates a poorer model fit, with a NS of 0 indicating that the model performs no better than a simple mean of the data.

3. The calibration R^2, i.e. the strength of the regression between measured DOC and each proxy (254 nm, phenolics, etc.).
4. Model R^2, i.e. the strength of the regression between measured and modelled DOC.
5. Root mean square error (RMSE) of the model regression.
6. Slope equation.

2.4. E ratio and SUVA assessment

Surface water was collected from four ditches in the Afon Ddu catchment on 25 occasions, from October 2010 to October 2012. Pore water was collected from four piezometers adjacent to each ditch on 23 occasions, from January 2011 to October 2012. The investigated ratios were the E2:E3 ratio (250 nm : 365 nm), E2:E4 ratio (250 nm : 400 nm), E4:E6 ratio (465 nm : 665 nm), and SUVA (254 nm : DOC).

2.5. Absorbance degradation experiment

In order to quantify the rate of absorbance degradation in stored samples, sixty five water samples were collected from ditches in the Afon Ddu catchment on one day in August 2012. Samples were stored in glass vials the dark at 4^{o}C and analysed within one day. Samples were filtered and analysed (without replication) for UV-Vis as previously described. After each analysis, samples were returned to storage and reanalysed every week for 12 weeks. One-way ANOVA was used to test for significant ($p < 0.05$) differences in absorbance between weeks. The data did not pass the Shapiro-Wilk test for normality, but ANOVA is considered to be relatively robust with non-normal data[63]. Levene's test was used to test for homogeneity of variances. Depending on the result of this test, the post-hoc test used was either Tukey HSD (equal variance assumed) or Tamhane's T2 (equal variance not assumed).

3. Results

3.1. DOC single wavelength proxy assessment

For all four sets of samples the best fit between DOC and absorbance occurred in the lower wavelengths, and declined as wavelength increased (Fig.1). R^2 was above 0.8 for some wavelengths in each of the four sample sets, indicating a strong correlation between DOC and absorbance. For pore water, overland-flow water, and Alwen Reservoir samples the strongest fit between absorbance and DOC (indicated by the highest R^2 and lowest residual variance) was found at 230 nm, but for ditch water the strongest fit was at 263 nm. Whilst the R^2 of the piezometer and Alwen Reservoir samples began dropping immediately at higher wavelengths, the R^2 for the other two sample sets were relatively stable up to approximately 350 nm, after which they declined. This decline in R^2 was a gradual process, and R2 dropped below 0.7 in ditch water samples at wavelengths above 702 nm, and above 474 nm in pore water. In overland-flow and Alwen Reservoir samples the R^2 dropped below 0.7 at 435 nm and 500 nm respectively. In the higher wavelengths (> 600 nm) the ditch, pore water and overland-flow samples show high variability in R^2 between adjacent wavelengths, but this is absent from Alwen Reservoir samples where R^2 continues to smoothly decline to 800 nm. Additionally, pore water samples show a ‘trough’ between 670 nm and 710 nm where R^2 rapidly decreases then increases, indicating a weaker fit between DOC and absorbance at these wavelengths. Examination of the raw spectra shows that there is a small increase in absorbance between these wavelengths.

3.2. Alternative DOC proxy comparison

The six methods detailed in section 2.2 were used to calculate DOC. Using the results from the DOC single wavelength proxy assessment, absorbance at 263 nm was chosen for ditch water, and 230 nm was chosen for pore water and stream water samples. Table 4 shows the summary results.

For ditch water the best estimates of DOC were found using an absorbance proxy at 254 nm, although the spectre area method and a proxy at 263 nm also performed well. The two wavelength model was also relatively accurate. An absorbance proxy at 400 nm and a phenolics proxy performed the least well. The highest Nash-Sutcliffe values were found for the spectra area method, and absorbance proxies at 254 nm and 263 nm, which all scored 0.96 (table 4). The lowest scoring method (0.86) was for the phenolics proxy. RMSE was lowest for an absorbance proxy at 254 nm, and this proxy also had the highest model R^2 (0.98, also the model R^2 for an absorbance proxy at 263 nm and the spectra area method). The model R^2 was lower for phenolic and 400 nm proxies (table 4). It should be noted that the wavelength that was selected on the basis of the DOC single wavelength proxy assessment (263 nm) generated identical calibration and model R^2 values as 254 nm. For ditch water there was variation between slope equations for the models (Fig.2); a perfect model should have an intercept at 0 and a slope of 1. The 400 nm proxy had an intercept furthest away from 0 (4.40 ± 0.78, $p < 0.01$), and the phenolics proxy had the closest (-0.58 ± 1.29, $p = 0.65$). The two wavelength method had a slope closest to 1 (0.96 ± 0.04, $p < 0.01$) and the 400 nm proxy had the one furthest away (0.77 ± 0.03, $p < 0.01$) (a full list of SEs and p values for gradients and intercepts is contained within the supplementary data, S1).

For pore water the best method was the two wavelength model, followed by absorbance at 254 nm and 230 nm. The next best method was the spectre area, followed by a proxy at 400 nm, then a phenolics proxy. The highest Nash-Sutcliffe value (0.92) was found for the two wavelength method and this method also had the lowest RMSE (3.89) (table 4). Considering Nash-Sutcliffe values and RMSE, the phenolics proxy performed the least accurately (respective values of 0.65 and 8.35), followed by an absorbance proxy at 400 nm (values of 0.77 and 6.76) (table 4). The results of the DOC single wavelength proxy assessment were only partially reinforced for pore water; the wavelength with the highest R^2 values from that analysis for pore water (230 nm) produced better calibration and model R^2 values for this experiment when compared to 254 nm, but 230 nm produced a marginally higher RMSE. Additionally, 230 nm and 254 nm both produced identical Nash-Sutcliffe values (table 4). When compared to slope equations for ditch water, pore water models had intercepts further removed from 0 (Fig.3). The largest difference was for the phenolics proxy (13.59 ± 2.91, $p < 0.01$) then a proxy at 400 nm (7.24, ± 2.71, $p = 0.01$), and the closest was the spectra area method (-0.93, ± 2.4, $p = 0.7$). Both the two wavelength method and an absorbance proxy at 254 nm produced the slopes closest to 1 (0.93, ± 0.04, $p < 0.01$, and 1.07, ± 0.05, $p < 0.01$ respectively). The phenolics proxy had a slope furthest from 1 (0.82, ± 0.06, $p < 0.01$).

For Alwen Reservoir catchment stream water the best models proved to be absorbance at 230 nm and the two-wavelength model. The next most accurate method was absorbance at 254 nm, followed by the spectre area method, then absorbance at 400 nm, and finally a phenolics proxy. Highest Nash-Sutcliffe values were found for the two wavelength model (0.98) and an absorbance proxy at 230 nm (0.95) (table 4). A proxy at 230 nm also had the lowest mean difference between measured and modelled DOC, the lowest RMSE, and a linear equation with an intercept closest to 0 (Fig. 4). A proxy at 400 nm performed poorly (relatively low Nash-Sutcliffe value, high RMSE, low R^2), but the phenolics proxy was even less accurate at estimating DOC concentrations (table 4).

3.3. E ratio and SUVA assessment

Overall means for all three E ratios were larger for pore water than ditch water, although this difference was only marginal for the E2:E3 and E2:E4 ratios (table 5). SUVA was higher for ditch water (table 5). Figures 5A and 5B shows the monthly data. For both ditch and pore water the E4:E6 ratio showed considerable variation over time, whilst the E2:E3 ratio was the most stable. There was evidence of shared trends in the E4:E6 ratio for both sample types, for example, the increase on the 5.7.11, and the peak on the 9.7.12. Regression analysis showed that the E4:E6 ratio was highly correlated between ditch and pore water ($R^2 = 0.91$), whilst there was no correlation between samples types for the E2:E3 ratio, E2:E4 ratio, or SUVA. Additionally, there was no relationship between pH and DOC concentration for ditch or pore water.

3.4. Absorbance degradation experiment

After twelve weeks of repeated measurements there was no consistent change in absorbance at any wavelength (Fig. 6). Instead, the mean absorbance displayed small fluctuations. The mean differences in absorbance between the original and week 12 measurements were extremely small (table 6). ANOVA showed that there were no significant differences between the original absorbance values and any of the later weekly measurements for 250 nm, 365 nm, 400 nm, 465 nm or 600 nm. At 400 nm, 465 nm and 600 nm there were significant differences in absorbance values between some weeks (Fig. 6) . Although the fluctuations in mean absorbance are of a larger absolute magnitude at 250 nm, and decrease with increasing wavelength, when expressed as a percentage of the mean absorbance they are similar for all wavelengths, though slightly higher at 600 nm (table 6).

For each sample, regression analyses were conducted between absorbance at every wavelength from 230 – 800 nm, and each of the weekly repeated measurements (table 7). Across all wavelengths the mean R^2 value for each sample was typically low, but three samples (samples 1, 8 and 61; table 7) had mean R^2 values > 0.3. However, these three samples displayed a positive relationship between absorbance and week (i.e. absorbance increased during storage). Furthermore, the individual R^2 values for these three samples at 254 nm (0.03, 0.25 and 0.45 for samples 1, 8 and 61 respectively) were lower than those at 400 nm (0.57, 0.56, and 0.83 respectively), suggesting that there was a more marked change in absorbance at 400 nm when compared to 254 nm. These changes in absorbance would translate into changes in DOC concentration of 0.3 – 6.1 mg L^{-1} (mean DOC of the three samples was 21.6 mg L^{-1}).

The limit of detection was estimated by running a blank ten times, and then calculating the standard deviation (table 8). The limit of detection was highest at either end of the UV-vis spectrum (254 nm and below, and 750 nm and above)

4. Discussion

4.1. DOC single wavelength proxy assessment

For all four data sets the fit between DOC concentration and absorbance wavelength was strongest in the shorter wavelengths, and declined as wavelength increased. This is expected; for humic acids absorbance decreases as wavelength increases and therefore using a shorter wavelength would give higher resolution[38], a finding also reinforced by Peacock *et al.*[42] who found that 254 nm was a more accurate proxy than 400 nm. For three of the sample sets the most robust proxy was 230 nm; the shortest wavelength investigated, whilst for ditch water samples 263 nm was found to be a marginally better predictor. It is probable that this difference is derived from the specific aromatic moieties comprising each sample's DOC, as numerous aromatic acids are present in natural waters[20]. Wavelengths such as 250 nm, 254 nm, and 280 nm have been associated with aromaticity[21,45] and a ratio of 253:203 nm has been shown to correlate with ester-, hydroxyl-, carbonyl- and carboxyl-substituted aromatic rings[18]. Despite this, Stedmon & Álvarez-Salgado[64] point out that there is still a knowledge gap concerning exactly what compounds are responsible for absorbance at different wavelengths. For ditch water and overland-flow samples the fit between DOC and absorbance was relatively stable in the short wavelengths, suggesting that numerous wavelengths between 230 nm and 350 nm would be equally suitable as DOC concentration proxies. This was not the case for pore water and Alwen Reservoir samples where R^2 decreased immediately as wavelength increased from 230 nm. This indicates that ditch water and overland-flow DOC contained a wide array of aromatic moieties which accordingly absorbed light in a wide range of the UV spectrum, whereas pore water and Alwen Reservoir samples had a much narrower aromatic fingerprint.

These findings call into question the use of longer wavelengths such as 400 nm as DOC proxies, and suggest that care should be taken when selecting a single wavelength as a DOC proxy. Although 230 nm gave the most accurate proxy for three out of the four sample sets here, it is inappropriate for certain waters, as NO_3^--N will interfere with absorbance at wavelengths shorter than 250 nm[38]. This is unlikely to have affected our results; highest measured nitrate concentrations in the Afon Ddu catchment during 2011 were 0.06 mg L^{-1} for ditch water and 0.31 mg L^{-1} for pore water (M. Peacock, unpublished data). The fact that different wavelengths were found to be accurate for pore water and surface water at the same site is not surprising, as Wallage & Holden[33] demonstrated that the relationship between absorbance and DOC changed with factors such as peat layer, due to changes in the proportions of differently-coloured compounds that comprise DOC. Clay *et al.*[65] noted similar changes after burning, where DOC concentration stayed the same but absorbance at 400 nm decreased as more years passed since the burn event.

To our knowledge, the style of the presentation of DOC single wavelength proxy data (i.e. individual R^2 values for regression between absorbance and DOC graphed against individual wavelengths) is seldom used, though Asmala *et al.*[41] have used it for estuarine water samples. As such, it offers a new way of visualising absorbance data and provides new insights into the quality of dissolved organic matter. For example, the ‘trough’ in the pore water samples between 670 nm and 710 nm shows an absorbance region where the relationship between DOC and absorbance is weaker than that immediately above and below this region. This suggests that between these wavelengths there is a compound that absorbs light but is not DOC. As the samples in question are pore water it seems logical that this is caused by some compound leaching into the water. It has been proposed that the boundary between oxic and anoxic peat layers is important in the formation of humic-iron colloids[66], and iron is known to contribute to water colour at 420 nm[67] as well as interfering with UV absorbance analysis[68]. The unknown compound interferes at much longer wavelengths however, and therefore is likely to be something different. Iron is present in the waters of the Afon Ddu catchment[69] and so the contaminant could be a colloidal ‘green rust’[70] such as Fe(II) Fe(III) hydroxyl carbonate, which has an absorbance peak at 650 nm[71]. Green rusts have been found in hydromorphic soils[72]. Iron hydroxide is another possibility, as it is common in natural waters[73] and absorbs light at 750 nm[61]. Other similar iron compounds may exist that could interfere with absorbance as these wavelengths. Another possibility is that the interference is simply due to some artefact of the analysis procedure. Without further evidence it is difficult to draw any robust conclusion.

4.2. Alternative DOC proxy comparison

For surface water the best DOC proxy was found to be absorbance at 254 nm as this displayed the highest Nash-Sutcliffe value (along with the spectra area method and a proxy at 263 nm) and had the lowest RMSE, therefore demonstrating the best model fit. For pore water the best model was the two wavelength model proposed by Tipping *et al.*[39] and advanced by Carter *et al.*[40] . For stream water the best DOC proxies were a single wavelength absorbance proxy at 230 nm, and the two wavelength method. . For both surface water and pore water, the phenolics proxy performed the worst, followed by an absorbance proxy at 400 nm.

These results are slightly dissimilar to those of Wang & Hsieh[38] who found that using the area under the spectra was a more accurate proxy than a single wavelength. The R^2 of the model calibrations was slightly higher for a proxy at 254 nm compared to one using the area under the spectra between 250 nm and 350 nm, and a proxy at 254 nm also demonstrated lower RMSE values for both surface and pore water. It may be that the difference can be explained by sample type; Wang & Hsieh[38] used commercially available humic acids whilst our analysis used samples from peatland catchments. It could be that some unknown difference between natural and commercial humic acids is responsible for the differing results, and it has been previously suggested that commercial humic acids are not true analogues of natural water samples[74]. Likewise, our results are partially at odds to those of Carter *et al.*[40] who found that using a two wavelength model increased R^2 by 0.02 or 0.05 when compared to a UV proxy at 270 nm or 350 nm. We report an increase in R^2 of 0.02 for pore water when using a two wavelength model rather than absorbance at 254 nm, alongside an increase in Nash-Sutcliffe value from 0.89 to 0.92. However, for surface water a single wavelength model using 254 nm produced a higher model R^2 compared to the two wavelength model by 0.01. Additionally, for surface water, RMSE for a 254 nm proxy was approximately half that of the two wavelength model. However, the two wavelength model did give a slope equation closest to a 1:1 line for surface water.

The results of the DOC single wavelength proxy assessment were echoed for this analysis; 230 nm, (the wavelength that was selected from that experiment for pore water and stream water), improved the R^2 values of the model and calibration when compared to a 254 nm proxy and had a lower RMSE. For stream water, 230 nm produced a slightly more accurate model. However, using the wavelength selected for surface water (263 nm) from the

DOC single wavelength proxy assessment produced a slightly poorer model when compared to 254 nm.

The data here support the conclusion of the DOC single wavelength proxy assessment and of Peacock *et al.*[42], in showing that 400 nm should be avoided as a DOC proxy. Absorbance at approximately 400 nm can be subject to interference by iron[67]. Additionally, the relationship between DOC and absorbance at 400 nm has been demonstrated to show considerable variation within the same catchment, according to changes in vegetation[75]. As such, using 254 nm as a proxy gave a higher R^2 value for both the calibration and testing of a DOC:absorbance model. Furthermore, the data here suggest that the use of phenolic compounds as a proxy may not be ideal, despite this method showing promise in an earlier study[42] It may be that the relationship between phenolics and DOC is open to more variation than the relationship between absorbance and DOC, or that phenolic concentrations may become slightly ‘decoupled’ from DOC concentrations due to factors such as vegetation changes or seasonality.

The two wavelength method of Tipping *et al.*[39] and Carter *et al.*[40] is clearly a useful DOC proxy. Using the model parameters described in their paper gave accurate predictions of DOC (see supplementary data, S2)), but small changes in the parameters improved the calculations for the site investigated. The parameters cited by Carter *et al.*[39] were generated using a large number of samples which were all taken from surface water, and as such were unsuitable for calculating DOC in pore water (S2). However, calibrating the model parameters using DOC and absorbance data then produced an excellent fit for pore water. This suggests that the model can potentially have widespread applicability for different types of water sample, provided a calibration dataset is available. As such, other researchers could potentially use our calibration dataset to calculate DOC concentrations in their pore water samples, in the absence of their own calibration dataset. Such a model may not be perfectly suited to other peatland systems, but it is not unknown for site-specific calibrations to be used elsewhere (e.g. Worrall *et al.*[37])

It has been previously suggested that small sample sizes can be used to establish calibrations to then calculate DOC concentrations from absorbance or phenolics[42]. The analysis here reinforces that point; DOC concentrations from just 18 water samples were used to create the various models for the Alwen reservoir catchment, and yet these models were quite accurate at predicting DOC in samples that were not used to create the model. However, it is important to consider that all three sample sets were created using data from just three or four different months, and were tested on data that was collected during three or four months. DOC concentration and composition varies seasonally, and as such the models may perform less accurately at other times of the year.

Finally, because the relationship between DOC and 400 nm has been observed to vary according to vegetation[75], a specific absorbance using 400 nm (as used by Worrall *et al.*[52]) is probably a useful metric to test for differences in DOC quality. This approach could be extended to other wavelengths, such as a specific absorbance at 600 nm, but more investigation is needed to determine if this is appropriate. For instance, in samples with low DOC concentrations, absorbance at 600 nm will be very low indeed, and may be indistinguishable from zero.

4.3. E ratio and SUVA assessment

Over approximately two years, the E2:E3 ratio was relatively stable and displayed small fluctuations within a narrow range of values for both ditch water and pore water, although it should be noted that the E2:E3 ratio often covers only a small ranges of values in natural waters (e.g. 3-6[45], 3-4.3[47]). This stability is somewhat due to the small difference between the two wavelengths used in the E2:E3 ratio. The E2:E4 ratio generally covers a wider range of values (e.g. 4-16[56], 14-16[46]) but this measure was also relatively stable during the course of our study. SUVA values are typically 0-6[21,47] and some temporal variation in SUVA was observed for ditch water. However, the E4:E6 ratio was subject to large changes through time, and values were consistent with those from the literature[20]. To some extent the fluctuations in all four ratios will be driven by both seasonal changes and weather events[76]. For example, storm events will contribute increased volumes of lower-DOC surface run-off into ditches, therefore diluting high-DOC ditch water[77]; the influence of storms on the E4:E6 ratio has been previously documented, with Grayson & Holden[78] showing that it generally declines during the first six hours of a storm. Field observations supported this hypothesis, with water in ditches being visibly more coloured during dry periods, and appearing clearer after heavy precipitation events (see supplementary data, S3, for relationship between rainfall and water colour). If absorbance at 400 nm (i.e. water colour) declines during a storm, this will result in a lower E4:E6 ratio. To compound this, storms may stimulate the release of suspended particles of sediment which may scatter light at longer wavelengths and further contribute to a lowered E4:E6 ratio. There is variation in the pore size of filters used in UV-vis analysis (0.2 – 1.6 µm)[57], and the choice of filter size will determine the particulate component (if any) of a water sample, thus hindering the reliability of direct comparisons of the E4:E6 ratio where different methods are used. The E4:E6 ratio was the only one of the four ratios examined where a relationship existed between ditch and pore water samples that were collected at the same time. This suggests that the E4:E6 ratio responded consistently to seasonal changes in surface water and pore water.

The E4:E6 ratio is often used as a measure of humification, and changes to it following peatland restoration have been cited as an indicator of biogeochemical changes[44,50]. The use of the E4:E6 ratio to determine restoration success appears questionable however, judging by the way it fluctuated at the study site where no anthropogenic changes were applied to the monitored ditches. It would only be possible to use the E4:E6 ratio to detect post-restoration changes if a long-term dataset was available, as this would allow seasonal changes to be observed. Because absorbance above 600 nm is generally low, it is possible for small changes in absorbance to strongly influence the E4:E6 ratio, and it is therefore important to regularly zero/blank the spectrophotometer. In agreement with our results, Park *et al.*[48] noted consistent E2:E4 ratios but varying E4:E6 ratios when comparing samples, and O'Driscoll *et al.*[56] found no relationship between the E4:E6 ratio and other common measures of DOC quality such as the E2:E3 ratio and fluorescence. They suggested that the E4:E6 ratio is not suitable to determine the structure of DOC, and Peuravuori & Pihlaja[45] proposed that the E4:E6 did not vary with aromaticity. Despite this, the fact that the E4:E6 ratio was highly correlated between surface water and pore water, and the fact that it fluctuated extensively over time, suggests that it is a useful metric to observe and measure seasonal and weather-driven changes in DOC quality. Because the low wavelengths used to calculate the E2:E3 and E2:E4 ratios were so highly correlated in our site data, it may be that they (and SUVA) are more suitable for detecting between-site differences in DOC quality, rather than temporal changes in DOC quality at individual sites.

The aforementioned tendency of iron to interfere with measurements around 400 nm[67] means that the accuracy of the E4:E6 ratio (and E2:E4 ratio) could be compromised in waters with high iron concentrations. Additionally, the presence of the unknown (probably iron-based) compound in pore water between 670-710 nm (shown in Fig.1 and discussed in section 4.1) could possibly interfere with the E6 measurement. We used 465:665 nm but 400:600 nm has been used (see table 2) as the E4:E6 ratio; this would remove the unknown interference at 665 nm, but reintroduce the interference of iron at 400 nm.

4.4. Absorbance degradation experiment

There was no consistent decrease or increase in absorbance for any wavelength after 12 weeks of repeated UV-Vis analysis on samples kept in dark and cold storage, and absorbance values after 12 weeks were remarkably similar to those measured at the start of the experiment. Interestingly, three samples showed small but consistent increases in absorbance during the period. The lack of degradation in absorbance across all samples is unexpected because, although not always explicitly stated, there is a general consensus that absorbance must be measured as soon as possible after water samples have been collected[57]. Carter *et al.*[40] noted a decrease of 5% after 50-120 days, and this value falls within the observed range of fluctuations found here. It is therefore plausible that the 5% value from Carter *et al.*[40] is not the result of a steady decrease in absorbance due to degradation, but is just a random fluctuation similar to those reported here, possibly due to instrument precision. Although their 5% figure is only mentioned in passing, it appears that this percentage was calculated from a one-off re-analysis of older samples, rather than a comprehensive temporally-repeated experiment. . The probable origin of the non-significant fluctuations in absorbance over the course of the experiment is human and machine error; for example, small discrepancies when pipetting samples for analysis. The limit of detection was typically low, but increased below 254 nm and above 750 nm. This is unlikely to pose a problem as absorbance is high at 254 nm, and wavelengths above 700 nm are seldom of interest in the study of peatland dissolved organic carbon.

It is important to state that there are caveats to this result. For example, calcium can cause DOC to come out of solution and to flocculate[79], resulting in a decrease in DOC concentration, and this can therefore be problematic where water samples are taken from rich fens. In such circumstances, absorbance must be measured before samples begin to flocculate.

An important inference from the lack of observed decrease in absorbance over time is that there must also be no change in DOC concentration. There is a substantial body of literature concerning sample preservation for marine environments, though some of it is contradictory. For instance, Sugimura & Suzuki[80] recommended filtration and cold storage, as they concluded that both freezing and acidification did not give reliable results. Contrary to this, Tupas *et al.*[81] suggested that cold storage (with or without filtration) resulted in a loss of DOC, and that freezing or acidification were preferable. There is less literature concerning non-marine systems but the US Environmental Protection Agency recommends that water utilities should preserve samples through filtration and acidification[57], and Spencer *et al.*[82] noted changes in absorbance following freezing and thawing of water samples from UK upland watercourses. The absence of an observed decrease in DOC concentration here suggests that filtration at 0.45 µm and cold storage in the dark was sufficient to preserve the samples from biological or chemical degradation. There are obvious caveats to this result; the samples were all collected on one day in August, and were all from the same site, therefore this result cannot be extrapolated to other water samples. Different sample types collected at other times of year from different peatland systems may not show this stability in absorbance, as it may depend on factors such as pH, or DOC concentration and composition. More research is clearly needed to determine how applicable this finding is and whether this stability in absorbance is a common property of peatland waters. . It is entirely feasible that if this experiment was performed again, a significant loss of absorbance might be observed. This could be the case if the DOC was less aromatic, and therefore subject to biological degradation. Additionally, the proportion of hydrophilic and hydrophobic components will influence the degradation of any sample[83]. Such properties will be intrinsically linked to factors such as seasonality, as this will influence the production and consumption of DOC.

Finally, disturbance such as fire can seemingly alter DOC composition[65] and might therefore also influence the stability of absorbance in a sample.

4.4. Conclusions

Out of the various DOC proxies that were investigated the phenolics proxy was the least accurate. Using absorbance at 400 nm was found to consistently perform with less accuracy when compared to 254 nm. Other wavelengths such as 230 nm and 263 nm also acted as robust proxies. The two wavelength method of Tipping *et al.*[39] and Carter *et al.*[40] was found to estimate DOC remarkably well for surface waters, and parameterisation further improved the model, although a single wavelength proxy at 254 nm also performed well. As such, the two wavelength method appears to offer a viable alternative to a single wavelength DOC proxy. If funds and equipment are available a calibration can be established to give the greatest accuracy possible. If direct DOC analysis for a calibration is not possible, then the 'universal calibration' parameters provided by Carter *et al.*[40] should still provide robust DOC estimations for surface water.

To monitor non-specific temporal changes in DOC quality, we suggest the use of the E4:E6 ratio as it displays systematic variations, although further work is required to interpret what these variations represent in terms of changes in DOC. The E2:E3 ratio, E2:E4 ratio, and SUVA may be more suitable for detecting changes in DOC quality between sites.

Finally, the results from the 12 week degradation experiment showed that absorbance, and therefore DOC concentrations, did not decline during storage for surface water samples collected on one sampling day. More testing is needed to determine how common this stability is to other sample types and sites.

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Table 1. List of wavelengths that have been used as proxies for DOC.

Wavelength (nm)	Reference
250	De Haan *et al.*[22]
254	Edzwald *et al.*[19]
260	Banoub[23]
270	Timperley[24]
280-400	Lawrence[25]
300	McKnight *et al.*[26]
320	Gorham[27]
330	Moore[28]
340	Tipping *et al.*[29]
355	Muller & Tankéré-Muller[30]
360	Collier[31]
365	Carpenter & Smith[32]
400	Wallage & Holden[33]
410	Hongve & Åkesson[34]
420	Fosberg[35]
436	Hongve & Åkesson[34]
450	Hongve & Åkesson[34]
465	Hautala *et al.*[36]
562	Carpenter & Smith[32]

Table 2. Details of various UV-vis measures used in the investigation of DOC composition.

Measure	Wavelengths (nm)	Reference
E2:E3 ratio	250:365	Peuravuori & Pihlaja[45]
E2:E4 ratio	252:452	Graham *et al.*[46]
	254:436	Selberg *et al.*[47]
	254:465	Park *et al.*[48]
E4:E6 ratio	400:600	Moore, 1987[49]
	450:650	Wilson *et al.*[44]
	460:660	Thurman[20]
	465:665	Wallage *et al.*,[50]
SUVA	254	Weishaar *et al.*[21]
	280	Duirk & Valentine[51]
	400	Worrall et al.[52]

Table 3. Details of when sampling took place for the model creation and testing for the alternative DOC proxy comparison. Ditch and pore water samples were from the Afon Ddu catchment, whilst stream samples were from the Alwen Reservoir catchment.

	Measured		Modelled	
	Month	No. samples	Month	No. samples
Ditches	Sept 2011	12	Oct 2011	11
	Dec 2011	12	Nov 2011	12
	Jan 2012	12	Aug 2011	12
	July 2012	12	Oct 2012	12
Total		48		47
Pore	Early July 2011	7	Late Sept 2011	10
	Late July 2011	9	Nov 2011	12
	Early Sept 2011	12	Aug 2012	12
	Oct 2011	12	Oct 2012	10
Total		40		44
Streams	Dec 2011	6	Oct 2011	5
	March 2012	6	Jan 2012	6
	May 2012	6	Feb 2012	5
			April 2012	6
Total		18		22

Table 4. Summary results for six different methods of indirectly calculating DOC in ditch and pore water samples from the Afon Ddu catchment, and stream samples from the Alwen Reservoir catchment. 1) the method of Carter *et al.*[40] that calculates DOC using absorbance at 270 nm and 350 nm, 2) the method of Wang & Hsieh[38] that uses the area under the UV spectra to create a calibration for DOC, 3) a calibration curve created using absorbance at 254 nm, 4) a calibration curve created using absorbance at 400 nm, 5) a calibration created using the optimum absorbance wavelengths (263 nm and 230 nm) derived from the DOC single wavelength proxy assessment, and 6) the method of Peacock *et al.*[42] that uses a calibration created using phenolic concentration. Mean diff is the mean difference between calculated and actual DOC, Nash-Sutcliffe value indicates how good the model fit is, calibration R^2 is the strength of the regression between each calibration of DOC and proxy. The method of Carter *et al.*[40] just uses absorbance to directly calculate DOC after model parameterisation and therefore has no calibration R^2. Model R^2 is the strength of the regression between measured and modelled DOC. Root mean square error (RMSE) is another measure of difference between modelled and measured DOC. *n* = 47 for ditch water, 44 for pore water, and 22 for stream water.

Ditch water	Abs 270/350 nm	Spectra area	254 nm	400 nm	263 nm	Phenolics
Mean diff (mg L^{-1})	1.69	1.69	1.49	2.78	1.51	2.57
Nash-Sutcliffe	0.94	0.96	0.96	0.89	0.96	0.86
Calibration R2	n/a	0.85	0.86	0.82	0.86	0.89
Model R2	0.97	0.98	0.98	0.93	0.98	0.91
RMSE	2.39	1.98	1.77	3.12	1.78	3.58

Pore water	Abs 270/350 nm	Spectra area	254 nm	400 nm	230 nm	Phenolics
Mean diff (mg L^{-1})	3.2	3.61	3.61	5.41	3.75	7.49
Nash-Sutcliffe	0.92	0.88	0.89	0.77	0.89	0.65
Calibration R2	n/a	0.68	0.72	0.55	0.75	0.47
Model R2	0.93	0.91	0.91	0.86	0.93	0.81
RMSE	3.89	4.8	4.76	6.76	4.79	8.35

Stream water	Abs 270/350 nm	Spectra area	254 nm	400 nm	230 nm	Phenolics
Mean diff (mg L^{-1})	1.04	1.29	1.16	2.24	0.97	4.85
Nash-Sutcliffe	0.98	0.92	0.93	0.78	0.95	0.19
Calibration R2	n/a	0.84	0.84	0.84	0.84	0.70
Model R2	0.97	0.94	0.95	0.87	0.96	0.89
RMSE	1.44	1.78	1.67	2.95	1.36	5.63

Table 5. Means, standard deviations, and ranges for the four ratios examined. *n* = 100 for ditch water, *n* = 86 for pore water, except for pore water SUVA where *n* = 85.

	Ditch water				Pore water			
	Mean	SD	Min	Max	Mean	SD	Min	Max
E2:E3	3.65	0.21	3.25	3.91	3.70	0.14	3.44	3.84
E2:E4	6.42	0.73	4.95	7.42	6.77	0.45	5.85	7.33
E4:E6	5.99	3.76	1.42	11.97	7.37	4.04	2.35	14.44
SUVA	4.58	0.89	3.36	6.41	4.00	0.47	3.44	4.77

Table 6. The mean difference between the original absorbance measurements and week 12 absorbance measurements, standard deviation, and the mean percentage deviation of weekly measurements when compared to the original measurements.

Wavelength (nm)	254	365	400	465	600
Mean difference	0.004	0.008	0.005	0	-0.001
SD	0.071	0.026	0.020	0.016	0.008
Mean % deviation	3.3	4.6	5	4.9	9.8

Table 7. Mean R^2 and standard deviations for regressions between absorbance and the weekly repeated measurements (week 0 – 13). For each water sample the R2 is the mean of 571 individual regressions at each wavelength from 230 – 800 nm.

Sample	Mean R^2	SD	Sample	Mean R^2	SD	Sample	Mean R^2	SD
1	0.48	0.20	23	0.13	0.12	45	0.20	0.18
2	0.17	0.02	24	0.04	0.05	46	0.18	0.16
3	0.04	0.03	25	0.02	0.03	47	0.05	0.02
4	0.10	0.11	26	0.01	0.01	48	0.11	0.08
5	0.07	0.06	27	0.02	0.02	49	0.07	0.08
6	0.20	0.15	28	0.15	0.09	50	0.26	0.27
7	0.03	0.03	29	0.20	0.18	51	0.13	0.14
8	0.52	0.12	30	0.03	0.03	52	0.10	0.07
9	0.24	0.14	31	0.17	0.10	53	0.10	0.08
10	0.11	0.05	32	0.07	0.04	54	0.09	0.08
11	0.13	0.07	33	0.26	0.08	55	0.17	0.15
12	0.22	0.05	34	0.06	0.07	56	0.05	0.04
13	0.00	0.00	35	0.07	0.05	57	0.06	0.05
14	0.12	0.08	36	0.13	0.10	58	0.17	0.11
15	0.09	0.04	37	0.06	0.05	59	0.06	0.03
16	0.08	0.05	38	0.21	0.14	60	0.10	0.06
17	0.25	0.10	39	0.16	0.07	61	0.75	0.10
18	0.06	0.04	40	0.24	0.21	62	0.15	0.18
19	0.03	0.02	41	0.24	0.23	63	0.26	0.15
20	0.11	0.09	42	0.09	0.09	64	0.06	0.09
21	0.08	0.06	43	0.14	0.08	65	0.15	0.17
22	0.05	0.04	44	0.10	0.11			

Table 8. The limit of detection at various wavelengths.

Wavelength (nm)	LOD
230	0.007
254	0.004
300	0.002
365	0.001
400	0.001
465	0.001
500	0.001
550	0.001
600	0.001
650	0.001
700	0.002
750	0.006
800	0.005

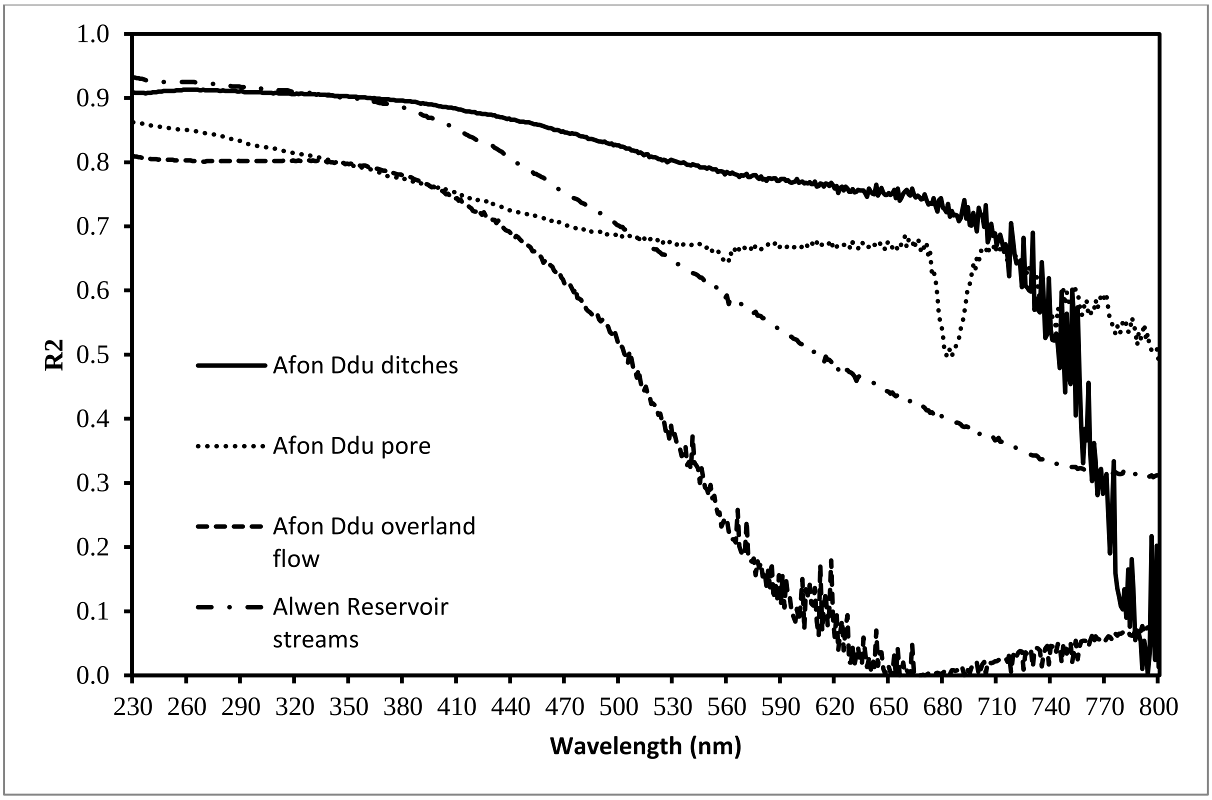


Figure 1. R^2 values for regressions between DOC concentration and absorbance for wavelengths between 230 nm and 800 nm for four sets of water samples. Afon Ddu ditch water *n*=108, Afon Ddu pore water *n*=98, Afon Ddu overland-flow water *n*=47, Alwen Reservoir stream water *n*=40.

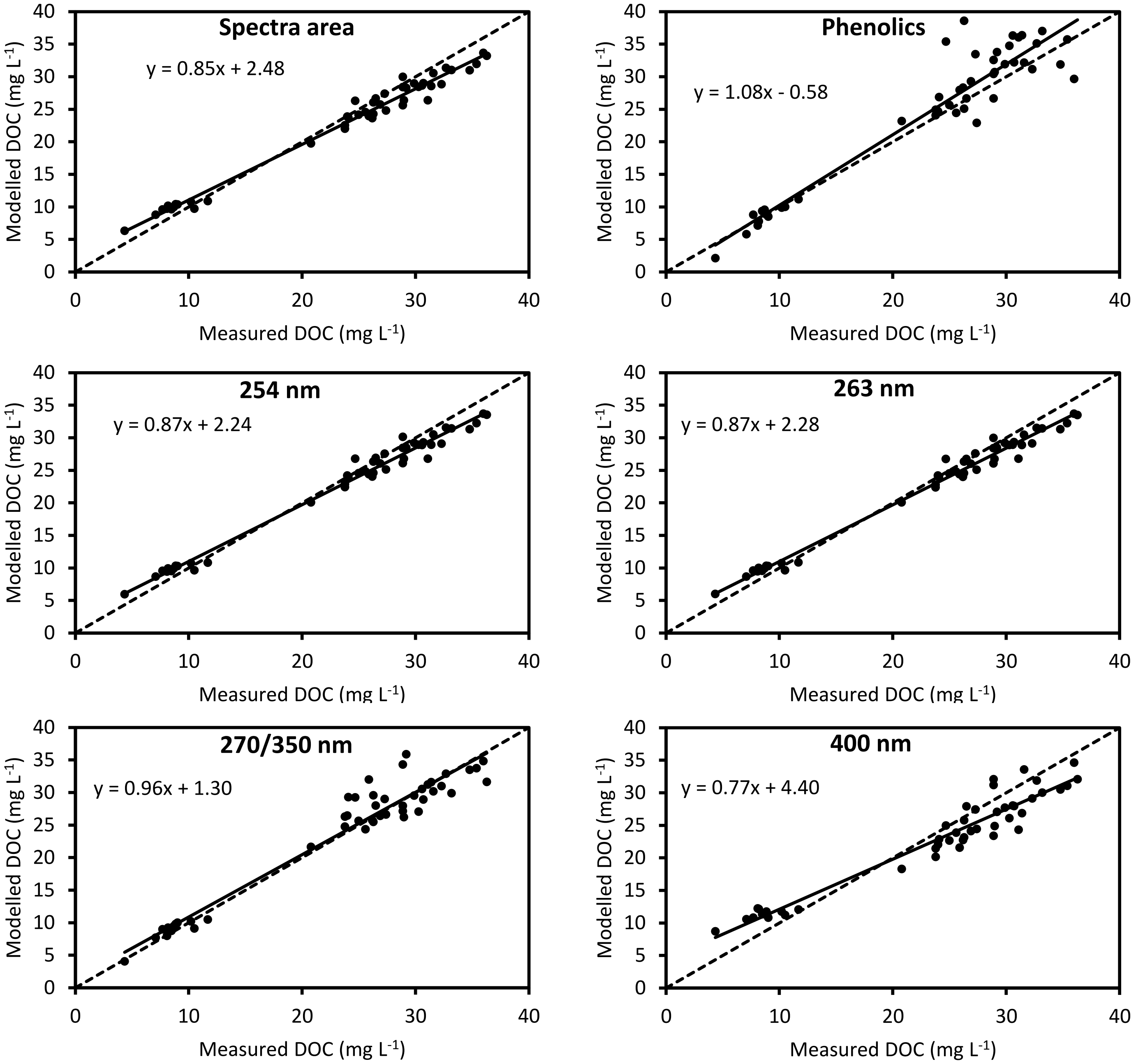


Figure 2. Surface water measured versus modelled DOC for each of the six DOC proxies, showing slope equations. Dashed line shows the 1:1 relationship. *n* =47. Model statistics are detailed in table 4.

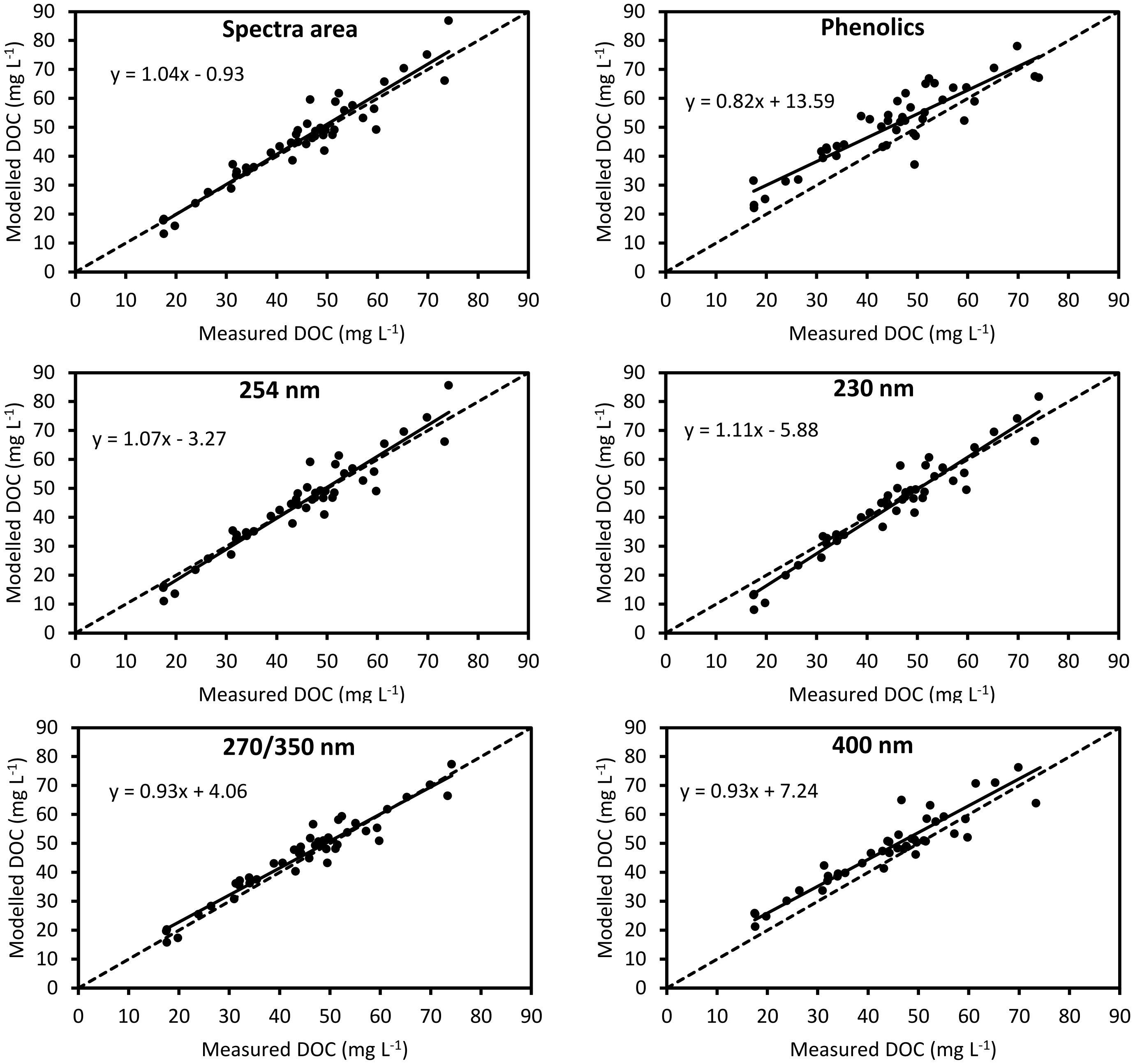


Figure 3. Pore water measured versus modelled DOC for each of the six DOC proxies, showing slope equations. Dashed line shows the 1:1 relationship. *n* =44. Model statistics are detailed in table 4.

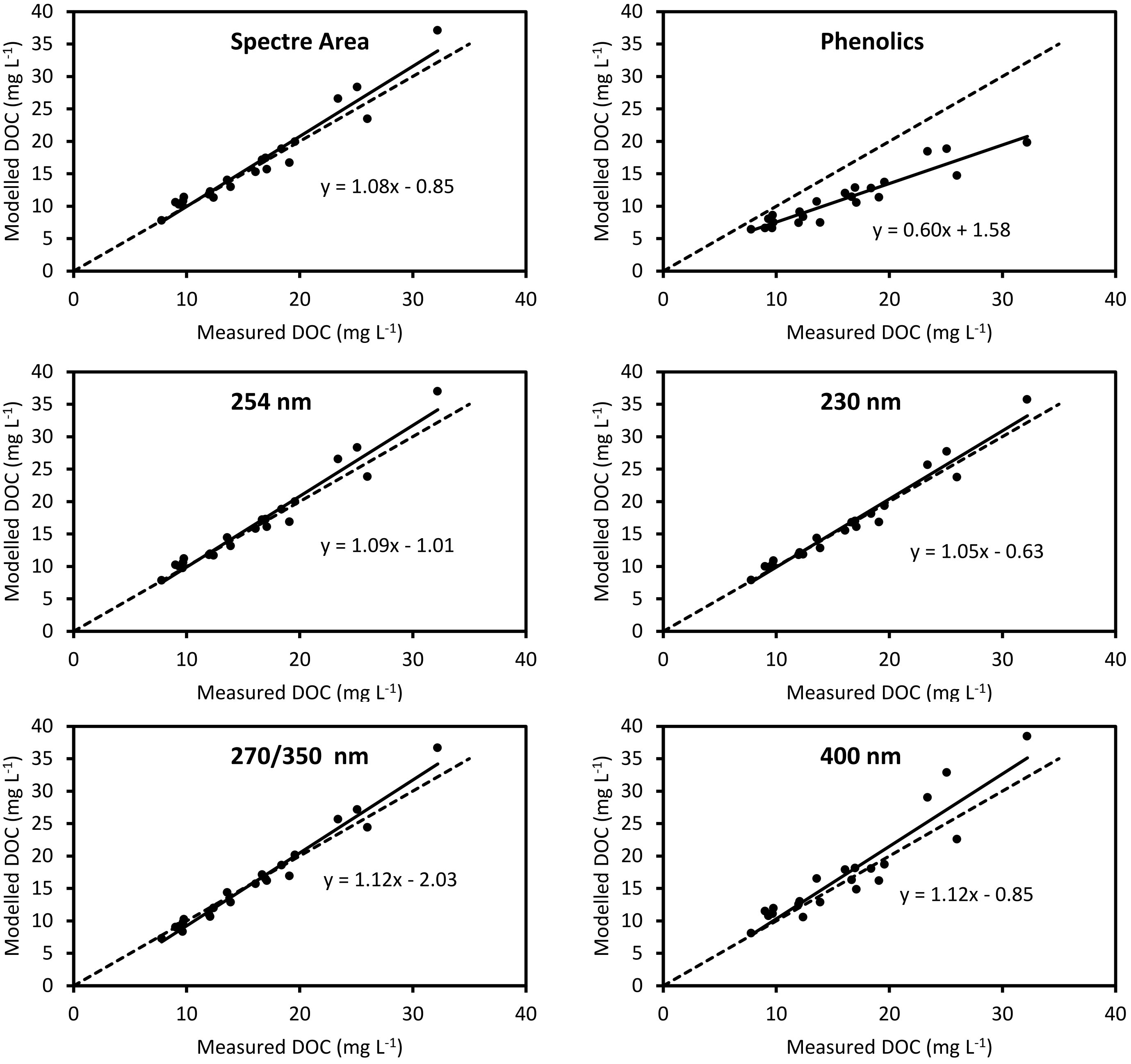
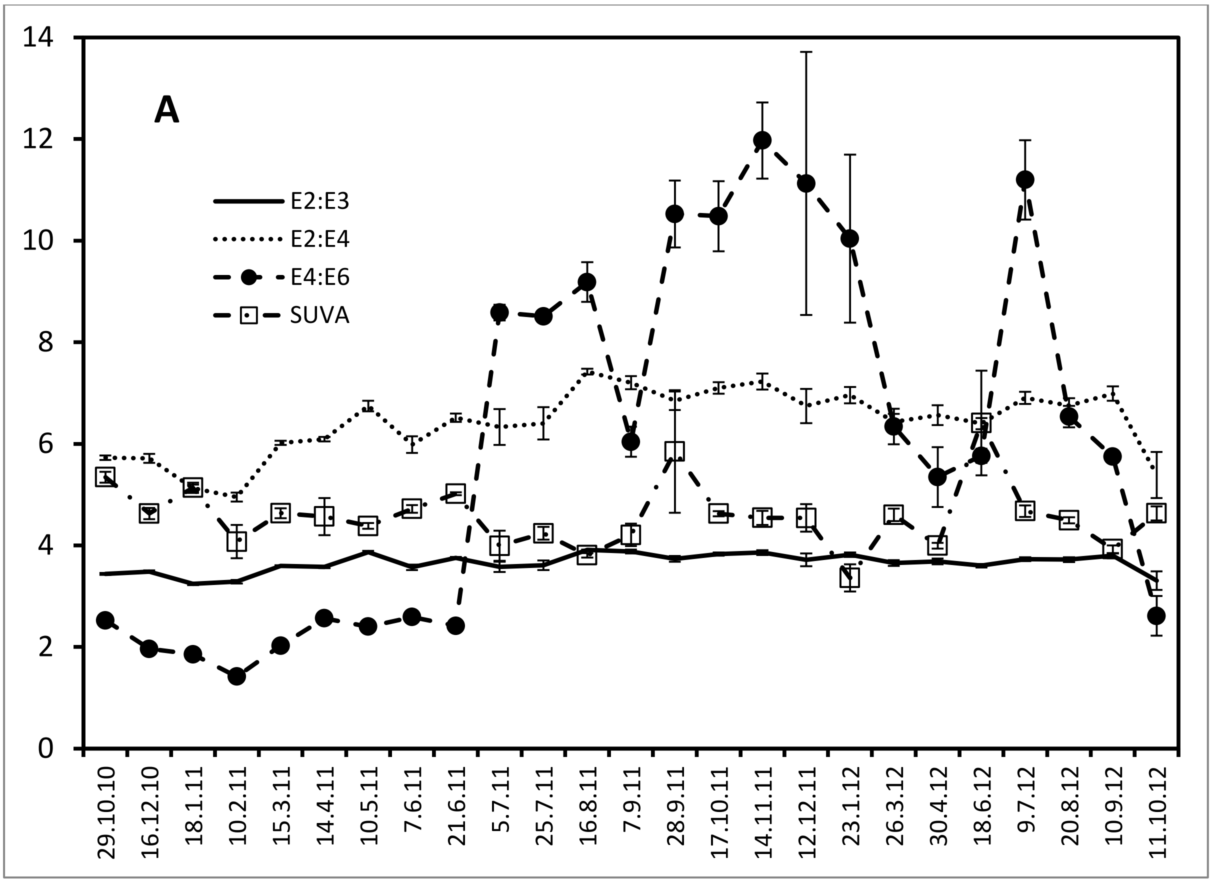
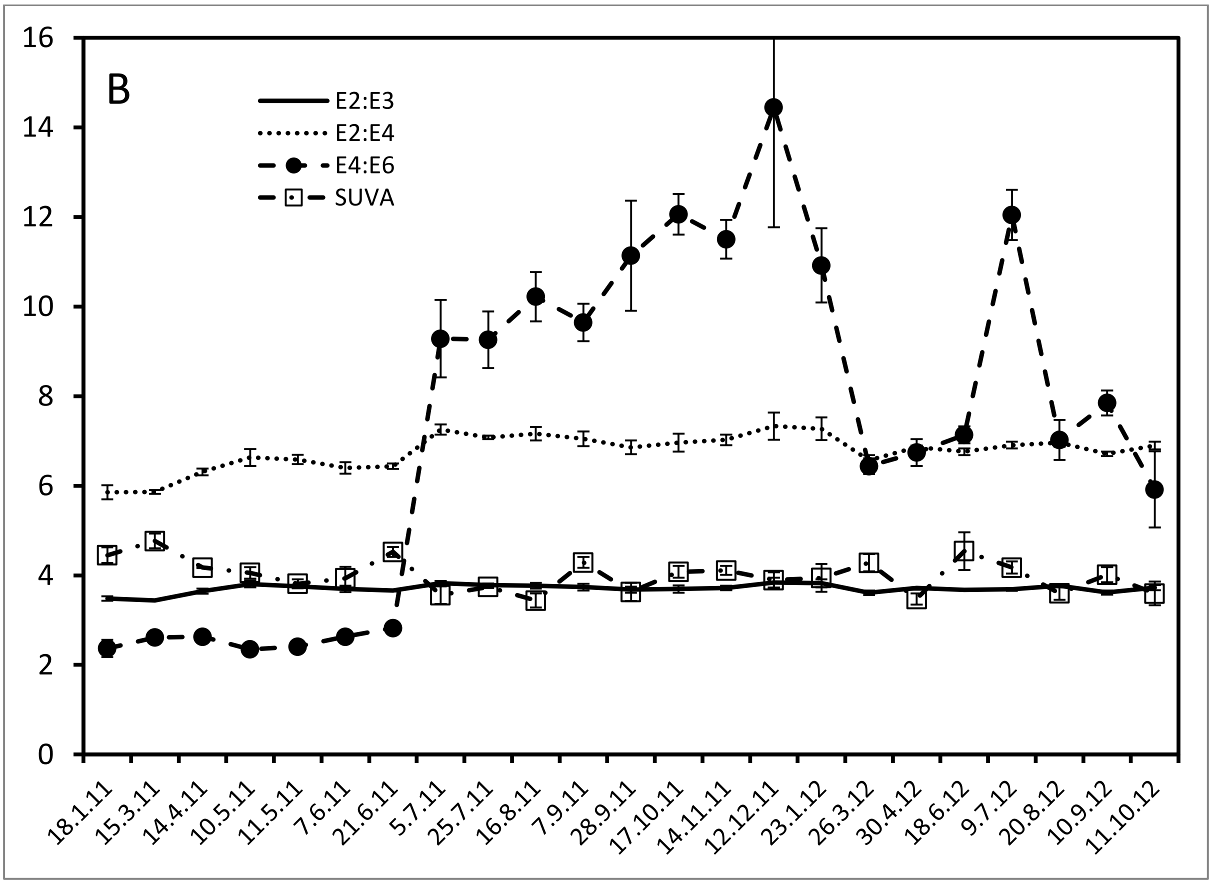


Figure 4. Stream water measured versus modelled DOC for each of the six DOC proxies, showing slope equations. Dashed line shows the 1:1 relationship. *n* =22. Model statistics are detailed in table 4.


A
E2:E3
E2:E4
E4:E6
SUVA
0
2
4
6
8
10
12
14
29.10.10
16.12.10
18.1.11
10.2.11
15.3.11
14.4.11
10.5.11
7.6.11
21.6.11
5.7.11
25.7.11
16.8.11
7.9.11
28.9.11
17.10.11
14.11.11
12.12.11
23.1.12
26.3.12
30.4.12
18.6.12
9.7.12
20.8.12
10.9.12
11.10.12

1163


B
E2:E3
E2:E4
E4:E6
SUVA
0
2
4
6
8
10
12
14
16
18.1.11
15.3.11
14.4.11
10.5.11
11.5.11
7.6.11
21.6.11
5.7.11
25.7.11
16.8.11
7.9.11
28.9.11
17.10.11
14.11.11
12.12.11
23.1.12
26.3.12
30.4.12
18.6.12
9.7.12
20.8.12
10.9.12
11.10.12

1164

Figure 5. Monthly mean values for E2:E3 ratio, E2:E4 ratio, E4:E6 ratio and SUVA for A) ditch water and, B) pore water. For each month *n* = 4, with the following exceptions for pore water: *n* = 3 for 5.7.11, 25.7.11, 10.9.12, 11.10.12 and *n* = 2 for 15.3.11. For pore water SUVA only, *n* = 3 for 11.5.11. Error bars show standard error of the mean.

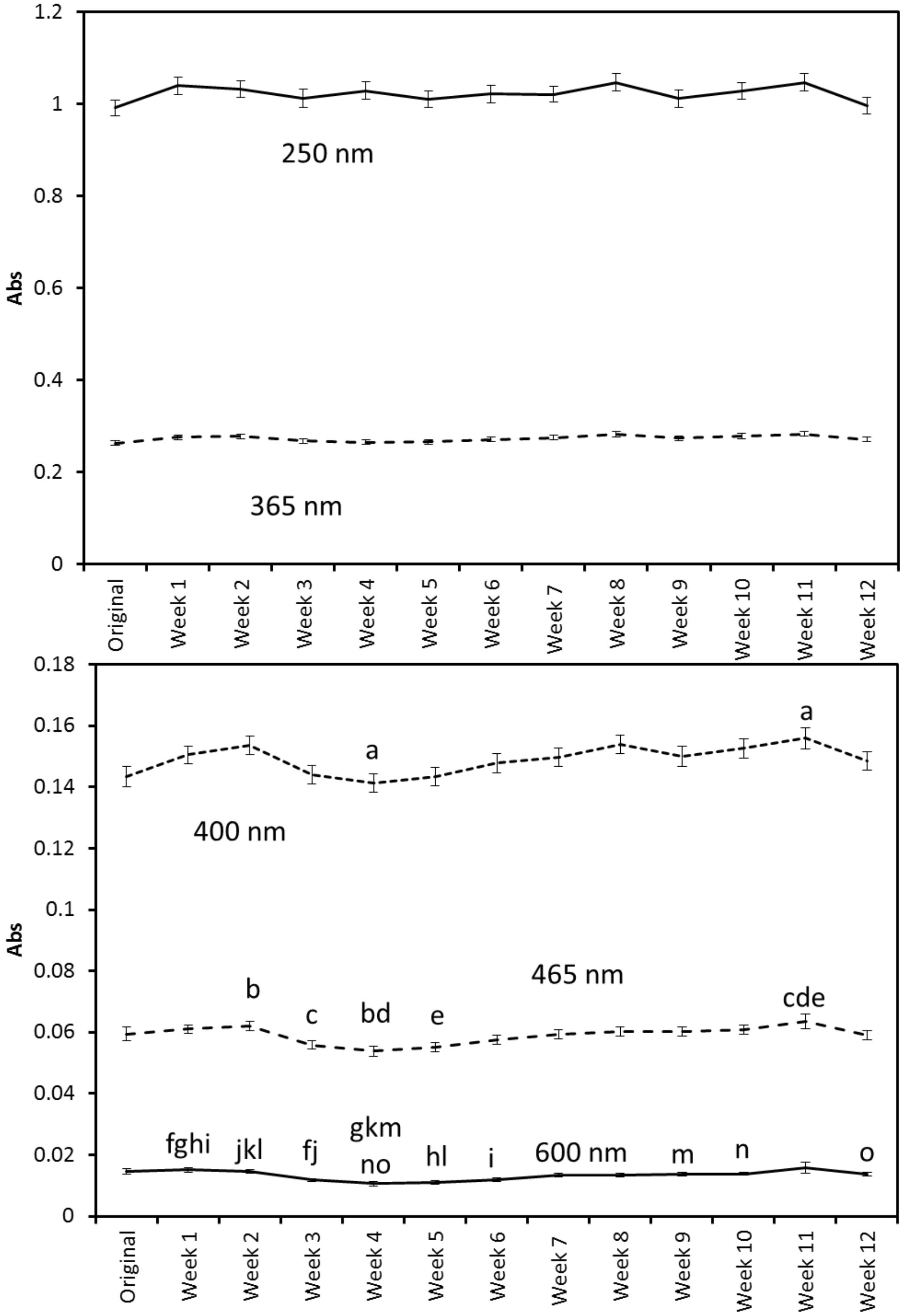


Figure 6. Mean absorbance values for five wavelengths, for a set of surface water samples (*n* = 65) that were analysed every week for twelve weeks. The wavelengths are 250 nm, 365 nm, 400 nm, 465 nm and 600 nm. Error bars show standard error of the mean. Letters show where ANOVA found significant ($p < 0.05$) differences between weeks. Post-hoc tests depended on whether equal variance was found for data at each wavelength. As such, Tukey HSD was used for 400 nm and 465 nm, whilst Tamhane's T2 was used for 600 nm. Note the difference in y axis scales.