Temperature quenching of CDOM fluorescence sensors: temporal and spatial variability in the temperature response and a recommended temperature correction equation.

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Abstract

Field-based instruments measuring chromophoric dissolved organic matter (CDOM) fluorescence are often used as a proxy for dissolved organic carbon concentrations in lakes and streams. CDOM fluorescence yield is, however, affected by water temperature at the time of measurement, a factor which varies on both diel and seasonal timescales. A temperature correction must therefore be applied to these data. We present data on temporal and site specific variability in temperature quenching of CDOM fluorescence for water from a humic lake and one of its main inflows in the west of Ireland. In addition, we present a temperature compensation equation and show that this equation is an improvement on methods previously proposed.

Introduction

Dissolved organic carbon (DOC) is one of the most important reservoirs of carbon in the biosphere. Chromophoric dissolved organic matter (CDOM) fluorescence is increasingly used as a proxy for DOC concentrations in streams and lakes. Concentrations of DOC can be highly variable on diel and seasonal timescales, especially in streams and rivers (Miller and McKnight 2010). Many studies have also reported increasing trends in DOC concentrations in surface waters over recent decades (e.g., Worrall 2007; Monteith et al. 2007). To better understand changes in CDOM at these scales of variability, collection of high frequency and long-term field data using CDOM fluorometers is desirable. However, CDOM fluorescence is quenched by increasing water temperature (Seredynska-Sobecka et al. 2007; Watras et al. 2011), a factor which itself varies on diel and seasonal timescales.

Watras et al. (2011) have recently proposed a temperature compensation method for CDOM fluorescence sensors using a temperature coefficient ρ, calculated as the ratio of slope to intercept for the regression of CDOM fluorescence on temperature. The same paper also reported that ρ was relatively constant for the two lake sites that they assessed, and relatively constant for consecutive monthly sampling dates over a summer period in those lakes. We present data on temporal variability in the degree of temperature quenching for both a humic lake and one of its main inflows in the west of Ireland. We also recommend that a dynamic temperature coefficient be used. We use this correction coefficient in a temperature correction equation which is subtly different to that used by Watras et al. (2011) but which produces a more successful temperature correction.

Methods and Procedures

In-situ monitoring of CDOM fluorescence was carried out at sites on Lough Feeagh, Co. Mayo, Ireland (53^{o} 56' 56" N, 9^{o} 34' 32" W) and on the Glenamong River, one of the two main inflows to the lake. Lough Feeagh is an oligotrophic, humic lake (area 4km², maximum depth 45m, a mean depth 14m, residence time 0.47 years). The DOC concentrations for Lough Feeagh and the Glenamong River (measured with a Sievers TOC Analyser model 5310, range 4ppb to 50ppm, accuracy ±2% or 5% ppb) for the period 13th July 2010 to 21st June 2011 and from 26th July 2010 to 4th June 2011 ranged from 7.7 to 12.3 mg L^{-1} and 3.6 to 21.5 mg L^{-1} respectively. The maximum and minimum daily water temperature recorded on sampling days in the Glenamong were 17.31^{o}C (July 2010) and 1.58^{o}C (December 2010) respectively. The corresponding values in Lough Feeagh were 16.69^{o}C (July 2010) and 3.88^{o}C (January 2011). The pH range for the same time period was from 6.1 to 7.1 and 4.0 to 6.7 for

100 Lough Feeagh and the Glenamong respectively. The annual rainfall for the region is
101 1500-2000mm. The altitude range for the Lough Feeagh catchment is 650m. The
102 Glenamong is a spate river, with flows returning to baseflow levels within hours after
103 precipitation events.

104 Two SeaPoint UV Fluorometers (from SeaPoint Sensors, Inc., Exeter, NH,
105 USA) were deployed on instrumented platforms, one in Feeagh (flow-through mode
106 at a depth of 1 metre) and one in the Glenamong River (flow-through mode
107 continuously submerged). The fluorometers use UV light emitting diodes (LEDs) as
108 the CDOM excitation source (Ex 370 nm CWL, 12 nm FWHM; Em 440 nm CWL, 40
109 nm FWHM, where CWL is the center wavelength and FWHM is the full width at half
110 maximum wave height). The gain was set to 1 for all measurements in the field and
111 laboratory. The instrument output was in mV and is referred to as relative
112 fluorescence units (RFU). RFU were converted to quinine sulphate units (QSU) based
113 on an instrument specific response. The relationship between RFU and QSU for the
114 CDOM fluorometer for the Glenamong River was QSU = 0.36 RFU, (r^2=0.94,
115 $p \leq 0.001$). The equivalent relationship for the CDOM fluorometer on Lough Feeagh
116 was QSU = 0.51 RFU, (r^2=0.98, $p \leq 0.001$). The range of RFU and QSU for the
117 Glenamong River from 12th January 2010 to 26th November 2011 was 54.1 to 442.2
118 RFU, and 22.8 to 121.9 QSU respectively. The range of RFU and QSU for Lough
119 Feeagh from the 5th March 2010 to 31st December 2011 was 74.5 to 337.7 RFU, and
120 36.4 to 77.5 QSU respectively. Water temperature was monitored by a Hydrolab
121 DateSonde 5X on Lough Feeagh and Hydrolab Quanta in the Glenamong River. All
122 parameters on the instrumented platforms, including CDOM fluorescence and
123 temperature, are measured at two minute intervals throughout the year. Data were
124 logged and stored by Campbell Scientific CR1000 data loggers at both locations.

125 There was also data from a continuous water level recorder OTT Hydromet (Orpheus
126 Mini) with a developed rating curve available for the Glenamong River.
127
128 Assessment of temperature quenching of CDOM fluorescence was carried out
129 using water samples collected on one occasion in each month between July 2010 and
130 June 2011 for both Lough Feeagh and the Glenamong River (Table 1). All water
131 samples were collected in the early morning from the sites. The CDOM fluorometers
132 were removed from the monitoring platforms for all temperature quenching
133 experiments which were conducted in the laboratory. Temperature quenching
134 experiments were started within one hour of sample collection. The fluorometer was
135 submerged in 12 L of unfiltered sample water in a Heto HMT 200 water bath and
136 heated steadily over two hours with constant stirring. All temperature quenching
137 experiments were performed in the dark. The temperatures was varied in each test
138 between 5°C and 24°C. CDOM fluorescence (mV) was recorded every minute using a
139 voltmeter. An Onset Tidbit temperature logger was placed in the bath to record the
140 temperature change over each experiment. The relationship between temperature and
141 fluorescence was plotted for each sampling occasion and the regression line
142 calculated. The regression lines for different months were compared using analysis of
143 covariance (ANCOVA) (Datadesk version 6.1) (Neter et al. 1996), with the null
144 hypothesis that there were no differences between slopes (Sokal and Rohlf, 1995).
145 Residual sum of squares were calculated for the difference between data corrected
146 using our temperature compensation equation (Eq. 6) and that presented in Watras et
147 al. (2011) (Eq.7). Data for Lough Feeagh and the Glenamong River were analysed
148 separately. All data were normally distributed.
149

150 Assessment of temporal and spatial variability in temperature quenching

151 There were strong negative linear relationships between temperature and CDOM
152 fluorescence for both sites on all sampling occasions (Fig. 1; Table 1). The coefficient
153 of determination (r^2) ranged from 0.957 to 0.996 (Glenamong) and 0.959 to 0.996
154 (Lough Feeagh) (Table 1). Both the intercept and the slope of these lines differed
155 between sampling occasions and between sites. The difference in the intercept
156 between sampling occasions would have reflected the difference in the concentration
157 of fluorescent DOC, while the differences in the slope would have reflected the
158 proportion of electrons that were fluorescing at any given time. These differences
159 indicate that the quantity and quality of DOC was changing from month to month and
160 between sites within the same month. The slope of the line ranged from -2.45 RFU
161 $^{o}C^{-1}$ to -6.64 RFU $^{o}C^{-1}$ for the Glenamong River and from -1.56 RFU $^{o}C^{-1}$ to -4.60
162 RFU $^{o}C^{-1}$ for Lough Feeagh. The slopes differed significantly between experiments
163 even when CDOM fluorescence was almost identical, for example, January 2011
164 (slope = -2.45 RFU $^{o}C^{-1}$; intercept = 214 RFU) and March 2011 (slope = -3.74 RFU
165 $^{o}C^{-1}$; intercept = 212 RFU) for the Glenamong River (ANCOVA, $p<0.0001$, F-ratio =
166 260.9, d.f. =1). In addition, on several occasions the slope was significantly higher
167 when the intercept decreased: examples here would be October 2010 and November
168 2010 for our river site (ANCOVA, $p<0.0001$, F-ratio = 1330.2, d.f. = 1), and
169 November 2010 and January 2011 (ANCOVA, $p< 0.0001$, F-ratio = 359.2, d.f. = 1)
170 for our lake site.

171 The ratio of slope:intercept, which was used as a temperature correction
172 coefficient by Watras et al. (2011), ranged in our experiments from -0.011 to -0.021
173 for the Glenamong River and from -0.011 to -0.025 for Lough Feeagh. The highest
174 values for this ratio for the river site were in November 2010 and in May 2011 (-0.021

175 in both cases). High ratio values were found in three sequential months, January,
176 February and March 2011 (-0.020 to -0.025), for Lough Feeagh. Watras et al. (2011)
177 reported a relatively consistent ratio for two different water sources for experiments
178 conducted during summer only. They noted that it might be necessary to carry out
179 additional temperature quenching assessments in very dynamic environments to
180 account for changes in organic matter quality or quantity. Our data, which included a
181 lake and a spate river and spanned a full annual cycle, support their suggestion that
182 repeated assessments are necessary. Variability in the temperature sensitivity of
183 fluorescence measured using excitation and emission wavelengths similar to those
184 used in our field instruments (excitation 300–340nm; emission 400–460nm) was also
185 reported by Seredynska-Sobecka et al. (2007), who investigated temperature
186 quenching of CDOM from a range of waters in the UK using emission excitation
187 matrices. They ascribed this variability to the presence of more than one fluorophore
188 at a given location in optical space.
189
190 Assessment of correction for temperature quenching

191 We used the results from our temperature quenching experiments to calculate
192 temperature corrected CDOM fluorescence values. We derived our correction
193 equation as follows, using a field temperature of 10^{o}C as an example (Fig 2):
194
195 $$d = a_2 - a_1 \qquad \text{Eq. 1}$$
196
197 where d = the required correction, a_1 = the measured CDOM fluorescence value,
198 $CDOM_{meas}$ (RFU), and a_2 = the CDOM fluorescence value corrected to a reference
199 temperature of 20^{o}C, $CDOM_{ref}$.

Since:

$$a_2 = a_3$$

$$d = (T_{ref}*m+C) - (T_{meas}*m+C) \quad \text{Eq. 2}$$

$$= (T_{ref}*m) - (T_{meas}*m) \quad \text{Eq. 3}$$

$$= m(T_{ref} - T_{meas}) \quad \text{Eq. 4}$$

where T_{ref} and T_{meas} are the measured water temperature and reference water temperature respectively (^{o}C), and m and C are the slope and intercept respectively of any given regression equation of temperature vs. CDOM fluorescence.

The slope, m, is expressed as a proportion of $CDOM_{meas}$ to give a temperature correction coefficient that allows the equation to be applied where CDOM concentration differs but where the equation of the line is the same:

$$f_t = m:(T_{meas}*m+C) \quad \text{Eq. 5}$$

where f_t is the temperature correction coefficient at temperature t.

We then used this coefficient in our temperature correction equation, (Eq. 6),

$$CDOM_{ref} = (CDOM_{meas} * (1 + f_t(T_{ref} - T_{meas}))) \quad \text{Eq. 6}$$

Our temperature correction equation differs in both the calculation of the temperature correction coefficient and in the form of the equation to that recently proposed by Watras et al. (2011). They calculated the temperature correction coefficient (*p*) as the ratio of the slope, m, to the intercept, C (CDOM fluorescence at a temperature of 0^{o}C). This value was then applied at all temperatures. Our dynamic temperature correction coefficient, in contrast, is the ratio of the slope to CDOM fluorescence based on the regression line equation and water temperature at the time of the field measurement. We compared data corrected using this equation to data corrected using the method of Watras et al. (2011) (Eq. 7).

$$CDOM_{ref} = (CDOM_{meas} / (1 + \rho(T_{meas} - T_{ref}))), \quad \text{Eq.7}$$

where $CDOM_{meas}$ is the measured CDOM fluorescence, T is temperature (°C), ρ is the temperature coefficient (slope:intercept), $_{ref}$ and $_{meas}$ are the measured and reference values.

We corrected two types of data with each equation (Fig. 3). The first data type was the uncorrected CDOM fluorescence data measured during the water bath tests for samples taken in January 2011 from both the Glenamong River and Lough Feeagh (Table 1 and Fig. 3 A and C). The second data type was synthetic CDOM data generated from the equation of the monthly regression line of CDOM fluorescence on temperature (Fig. 3 B and D). In both cases, the effect of increasing temperature was fully removed by Eq.6 only, that is the slope of the line from the regression for the data corrected using Eq. 6 was not significantly different from zero (Table 2). The

248 RSS was also consistently lower for data from the water bath tests corrected using
249 Eq.6 than for that corrected using Eq.7 (Table 1).

250 The difference in the CDOM fluorescence data corrected using Eq. 6 as
251 opposed to Eq. 7 is due to both the form of the equation and to the use of the dynamic
252 temperature correction factor. Using the relationship between CDOM fluorescence
253 and temperature for the Glenamong River for March 2011 (Table 1) as an example,
254 the relative effect of these will differ depending on temperature (Table 3). At a
255 temperature of 0^{o}C, $f = p$ and therefore the difference in the corrected value is entirely
256 due to the effect of the difference in form of the two equations. The relative effect of
257 using f rather than p increases at temperatures closer to the reference temperature.
258 Based on the relationship between RFU and QSU established for the Glenamong river
259 described above, the relationship between QSU and DOC (r^2= 0.60, $p \leq 0.001$, n=319),
260 the concentration (mg L^{-1}) of DOC = 0.11 QSU and 0.6 (r^2 = 0.6, $p \leq 0.001$), the
261 difference at 0^{o}C would be 19.4 RFU, 7.0 QSU and 1.4 mg DOC L^{-1} respectively,
262 while that at 10^{o}C would be 11.2 RFU, 4.0 QSU and 1.0 mg DOC L^{-1}.

263 A comparison of data from a field deployment of the CDOM fluorometer from
264 the 25th to the 27th of March 2011 from the Glenamong River corrected using Eq.6
265 and Eq.7 showed that the values using Eq.6 differed by up to 64 RFU from
266 uncorrected CDOM measurements, while those corrected using Eq.7 differed by 46
267 RFU from uncorrected CDOM measurements (Fig. 4). We also highlight that the
268 potential implications of the difference in correction methods for temperature
269 quenching, or indeed not correcting for temperature quenching at all, can be much
270 greater in deployments in rivers and streams such as the Glenamong when compared
271 to deployments in lakes, due to the larger diel range in water temperatures in the
272 former. The largest diel temperature range on the dates when water samples were

taken from the Glenamong River, for example, was 9.65^{o}C (May 2011). In contrast, the largest temperature range for Lough Feeagh was only 3.06^{o}C (June 2011).

Recommendations

- Our results establish that the temperature quenching effect on CDOM fluorescence can be highly variable. The relationship had a seasonal pattern in our lake site, Lough Feeagh, with higher temperature correction coefficients in January, February, and March 2011. There was no seasonal pattern in the temperature quenching effect at our spate river site, although the results were variable. We suggest that the temperature coefficient should be assessed on at least a seasonal basis in lakes and more often in rivers and streams.
- We recommend that the temperature correction coefficient be calculated as a function of water temperature at the time of measurement as in Eq. 5, rather than as a simple ratio between the slope and intercept.
- We have shown that Eq. 6 is successful at eliminating the temperature quenching effect and we recommend its use to correct CDOM fluorescence data.

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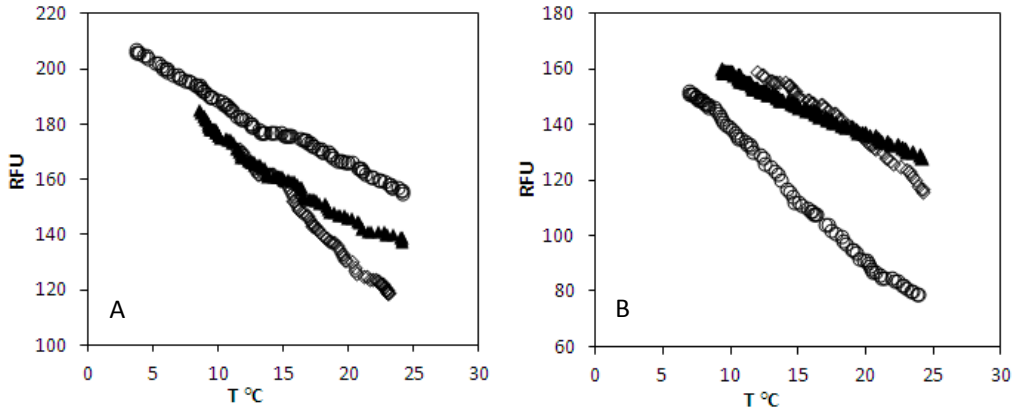

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318 Figure 1: Examples of the relationship between temperature (^{o}C) and CDOM

319 fluorescence (RFU) for the Glenamong River (A) and Lough Feeagh (B): November

320 2010 (open diamonds), December 2010 (filled triangles), and January 2011 (open

321 circles). Slopes and intercepts for these lines are given in Table 1.

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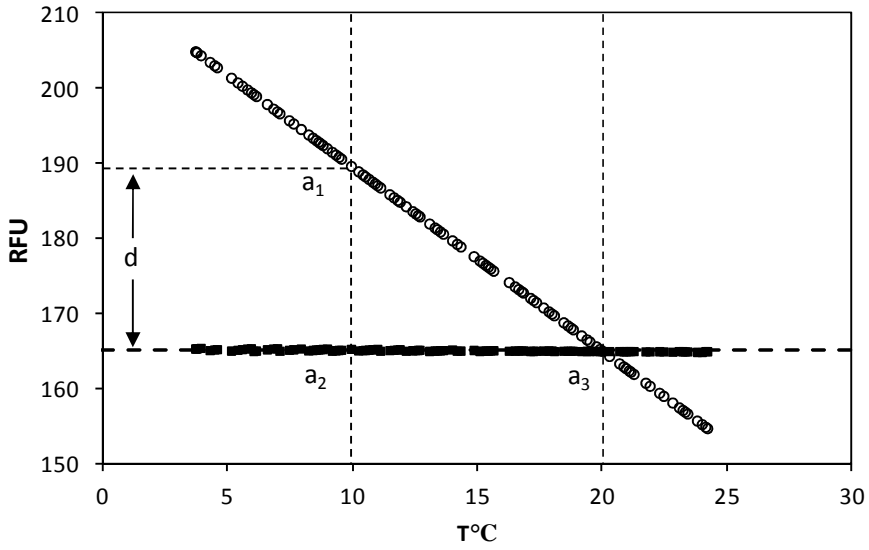


Figure 2: Derivation of the equation for calculation of the required temperature correction, d (RFU), using a field temperature of 10°C as an example.

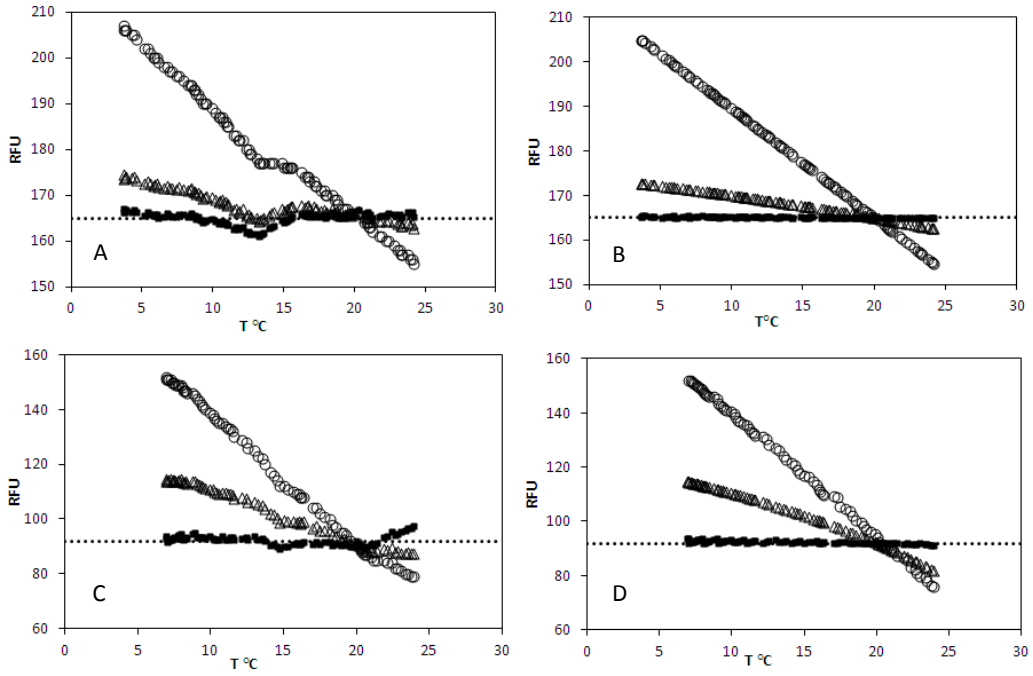
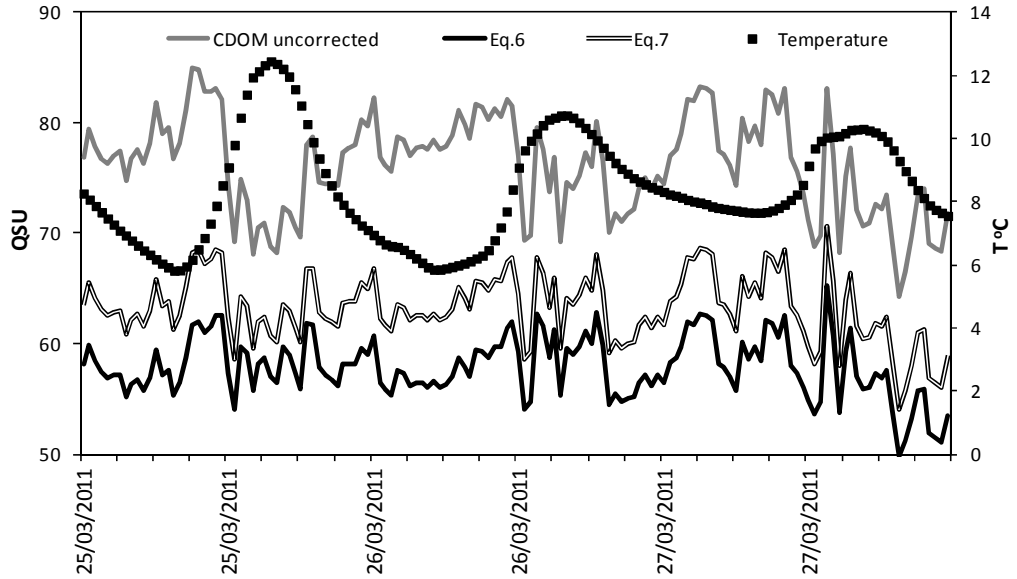


Figure 3: A. and C: uncorrected CDOM fluorescence data (RFU) generated from the slope and intercept for the January 2011 water bath test measured at a range of temperatures for the Glenamong River (A) and Lough Feeagh (C), January 2011 (open circles); these data corrected to 20°C using the method proposed in Eq.6 (filled squares); and these data corrected to 20°C using the method of Watras et al. (2011) in Eq.7 (open triangles) and; B and D: synthetic CDOM fluorescence data (RFU) generated from the slope and intercept for the January 2011 water bath test for the Glenamong River (B) and Lough Feeagh (D) (open circles); these data corrected to 20°C using the method proposed in Eq.6 (open squares) and these data corrected to 20°C using Watras et al. (2011) in Eq.7 (open triangles); reference fluorescence (dotted line).



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365 Figure 4: Uncorrected CDOM fluorescence data (QSU) from the Glenamong River,
366 25th of March 2011 to 27th of March 2011, 30 minute mean values (grey line); these
367 data corrected using Eq. 6 (black line) and corrected using Eq. 7 (double black line);
368 water temperature, black square.

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Table 1: Slope and intercept for the regression of temperature on CDOM fluorescence for the Glenamong River and Lough Feeagh; coefficient of determination (r^2) for the line; temperature coefficient, ρ, calculated as slope:intercept (Watras et al. 2011); our temperature correction coefficient *f* calculated as slope:fluorescence at two example temperatures of 10°C (f_{10}) and 15°C (f_{15}); residual sum of squares (RSS) based on Eq.6 and Eq. 7; Monthly average DOC mg L^{-1}.

	Slope	Intercept	r^2	*p*	f_{10}	f_{15}	RSS Eq.6	RSS Eq.7	**DOC mg L^{-1}
Glenamong									
26-Jul-10	-3.46	329	0.987	-0.011	-0.012	-0.012	26.6	134.1*	12.43
18-Aug-10	-3.78	329	0.996	-0.011	-0.013	-0.014	188.3	2802.2*	10.6
24-Sep-10	-5.33	320	0.993	-0.017	-0.020	-0.022	115.6	2361.8*	8.47
12-Oct-10	-2.50	250	0.985	-0.010	-0.011	-0.012	148.9	739.6*	8.86
15-Nov-10	-4.60	224	0.988	-0.021	-0.026	-0.030	227.5	4883.2*	7.93
08-Dec-10	-2.95	205	0.980	-0.014	-0.017	-0.018	336.9	3489.7*	6.08
03-Jan-11	-2.45	214	0.987	-0.011	-0.013	-0.014	206.9	1849.0*	7.31
24-Feb-11	-3.21	245	0.991	-0.013	-0.015	-0.016	139.4	1434.5*	8.37
28-Mar-11	-3.74	212	0.957	-0.018	-0.021	-0.024	459.1	2331.7*	7.05
27-Apr-11	-2.67	226	0.990	-0.012	-0.013	-0.014	49.3	334.4*	7.03
20-May-11	-6.64	321	0.994	-0.021	-0.026	-0.030	274.7	16242.2*	10.68
20-Jun-11	-4.39	286	0.994	-0.015	-0.018	-0.020	88.6	1749.3*	7.61
Lough Feeagh									
13-Jul-10	-2.47	156	0.989	-0.016	-0.019	-0.021	137.8	3828.6*	8.46
19-Aug-10	-1.72	163	0.972	-0.011	-0.012	-0.013	23.3	80.1*	8.60
16-Sep-10	-1.98	138	0.996	-0.014	-0.017	-0.018	12.4	1287.3*	8.73
08-Oct-10	-2.58	196	0.994	-0.013	-0.015	-0.016	27.6	270.6*	8.66
15-Nov-10	-3.38	201	0.986	-0.016	-0.019	-0.021	134.1	1552.0*	8.48
08-Dec-10	-2.13	179	0.994	-0.012	-0.014	-0.014	40.6	1117.0*	10.05
10-Jan-11	-4.60	184	0.995	-0.025	-0.033	-0.040	268.1	17119.1*	10.08
22-Feb-11	-4.53	193	0.991	-0.023	-0.031	-0.036	550.7	8685.1*	9.27
28-Mar-11	-3.45	174	0.959	-0.020	-0.025	-0.028	595.3	4286.3*	8.54
28-Apr-11	-1.56	145	0.992	-0.011	-0.012	-0.013	17.3	143.3*	8.86
17-May-11	-2.89	165	0.961	-0.018	-0.021	-0.024	405.1	1591.7*	8.15
02-Jun-11	-3.87	171	0.973	-0.023	-0.029	-0.034	150.1	6646.1*	8.52

*Significantly different at p=0.05

**Based upon monthly average DOC samples taken during the study period.

390 Table 2: Comparison of the regression of temperature on temperature corrected

391 CDOM fluorescence, corrected using Eq.6 and Eq.7 for the Glenamong River and

392 Lough Feeagh: data from January 2011.

	Equation	Variable	Coefficient	s.e of coefficient	t-ratio	prob	r^2
Glenamong							
Uncorrected	Eq.6	Temp	0.02	0.02	0.75	0.45	0.5
	Eq.7	Temp	-0.47	0.02	-21.8	≤0.0001	81.9
Glenamong							
Synthetic	Eq.6	Temp	0.00	0.00	0.0	1.00	0.0
	Eq.7	Temp	-0.49	0.00	-175.0	≤0.0001	99.7
Feeagh							
Uncorrected	Eq.6	Temp	0.04	0.04	1.12	0.26	1.5
	Eq.7	Temp	-1.84	0.03	-72.6	≤0.0001	98.5
Feeagh							
Synthetic	Eq.6	Temp	0.00	0.00	0.00	1.00	0.0
	Eq.7	Temp	-1.85	0.02	-92.8	≤0.0001	99.0

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405 Table 3: Measured CDOM fluorescence (RFU) at four temperatures (0, 5, 10, and
406 15°C) corrected using i) Eq. 6, ii) Eq. 7 and iii) Eq. 6 but applying *p* instead of *f*. The
407 total difference between CDOM fluorescence data corrected using Eq. 6 and Eq. 7 is
408 quantified in RFU, QSU, and mg DOC L^{-1}. The percentage difference due to the form
409 of the equation, and to use of *f*, the dynamic temperature correction factor as opposed
410 to *p*, the constant temperature correction factor is also quantified.

Temp	Meas		Cor Equ. 6		Cor Equ. 7	Cor Equ. 6 with *p*	total diff	total diff	total diff	% diff due to equation	% diff due to *f*
°C	RFU	*f*	RFU	*p*	RFU	RFU	RFU	QSU	mg DOC L^{-1}		
0	212.0	-0.0176	137.2	-0.0176	156.7	137.2	19.5	7.0	1.4	100	0
5	193.3	-0.0193	137.2	-0.0176	152.9	142.1	15.7	5.6	1.2	68	32
10	174.6	-0.0214	137.2	-0.0176	148.4	143.8	11.2	4.0	1.0	41	59
15	155.9	-0.0240	137.2	-0.0176	143.3	142.1	6.1	2.2	0.8	18	82

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