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A temperature compensation method for CDOM fluorescence sensors in freshwater

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Abstract

The effect of temperature on CDOM fluorescence was investigated in dystrophic freshwaters of Wisconsin and in aqueous standards. Laboratory experiments with two commercial in situ fluorometers showed that CDOM fluorescence intensity decreased as ambient water temperature increased. A temperature compensation equation was derived to standardize CDOM fluorescence measurements to a specific reference temperature. The form of the equation is: $CDOM_r = CDOM_m/[1 + \rho(T_m - T_r)]$, where T is temperature (°C), ρ is the temperature-specific coefficient of fluorescence ($°C^{-1}$), and the subscripts r and m stand for the reference and measured values. (We note that an analogous function is used widely to calculate temperature-specific conductance from the measured conductivity of natural waters.) For the two sensors we tested, the temperature-specific fluorescence coefficients (ρ) were –0.015 ± 0.001 and –0.008 ± 0.0008 for Wisconsin bog waters at 20°C. When applied to field data, temperature compensation removed the effect of multi-day trends in water temperature, and it also damped the diel CDOM cycle. We conclude that temperature compensation is a necessary and important aspect of CDOM monitoring using in situ fluorescence sensors.

As interest in the aquatic cycle of organic carbon (OC) has increased, the deployment of in situ optical sensors to measure CDOM fluorescence (chromophoric dissolved organic matter) as a proxy for OC concentration has become more common (e.g., Downing et al. 2009; Sandford et al. 2010). CDOM sensors typically use UV light (~350 nm) to excite the emission of blue light (~450 nm) from certain organic fluorophores, allowing investigators to distinguish CDOM from more commonly measured phytoplankton pigments. Given that CDOM may be more labile than previously thought and given that rates of OC mineralization may vary with fluctuating environmental factors, such as temperature and light, these inexpensive sensors could afford a substantial advantage over traditional wet chemistry methods—provided that the artifactual effects of environmental factors on fluorescence efficiency are well constrained (Graneli et al. 1996; Bertilsson and Tranvik 2000; Bastviken et al. 2004; Hanson et al. 2003; Vahatalo 2009).

Here, we quantify the effect of temperature on the fluorescence of CDOM from two dystrophic Wisconsin lakes and an aquatic NOM reference material. Based on laboratory experiments over a wide range of OC concentrations, we derive a function that can be used to standardize CDOM measurements to any reference temperature (and, thereby, remove the effect of temperature variation on CDOM fluorescence). Using a reference temperature of 20°C, we then apply the function to field data and show how temperature compensation affects temporal changes in CDOM fluorescence under natural conditions.

Methods and procedures

Two commercial CDOM fluorometers were used: 1) the C3 Submersible Fluorometer from TurnerDesigns, Inc.; and 2) the

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SeaPoint UV Fluorometer from SeaPoint Sensors, Inc. Both fluorometers use UV LEDs as the CDOM excitation lamp. Fluorescence specifications for the SeaPoint sensor were Ex 370 nm CWL, 12 nm FWHM; Em 440 nm CWL, 40 nm FWHM. For the C3, the fluorescence specifications were Ex 340 nm CWL, 110 nm FWHM; Em 470 nm CWL, 60 nm FWHM (where CWL is the center wavelength, and FWHM is the full width at half maximum wave height).

The study sites were two wetland-dominated lakes in northern Wisconsin: Crystal Bog (CB) and Trout Bog (TB). The bogs are situated in *Sphagnum*-dominated sub-catchments of the Trout Lake watershed (46°N, 89°W) roughly 5 km apart (http://www.lter.limnology.wisc.edu). Both have moderately tea-stained water, with DOC concentrations ranging from ~10 to ~20 mg C L^{-1}, depending on the season and antecedent weather conditions. For field monitoring, the CDOM sensors were submerged at a depth of ~0.5 m below the GLEON buoy in the center of the CB lake (www.gleon.org). The instrumentation buoy measured ~1.2 m × ~1.7 m, and it provided 12 VDC power and data-logging capability for the fluorometers. Data were collected continuously at 10 min intervals for successive time periods of 2 to 4 weeks during spring and summer. Laboratory experiments were conducted between field deployments, using sequential dilutions of CB and TB lake water. Laboratory experiments were also conducted with reconstituted Suwannee River NOM (IHSS aquatic reference material #1R101N; www.ihss.gatech.edu/) and with a quinine sulfate solution (Sigma-Aldrich).

For laboratory experiments at Trout Lake Station, the sensors were submerged in 5-L glass beakers that contained 4 L of the experimental lake water or aqueous reference material. The beakers with water and fluorometers were first cooled to ~5°C in a dark refrigerator and then transferred to a dark incubator where they gradually warmed to ~30°C over a period of 4 to 5 h (with constant stirring). Black cloth was placed under the beakers in the incubator to minimize light reflection. CDOM fluorescence and water temperature were simultaneously logged at 1-min intervals as the beakers warmed. For each set of lab experiments, the lake water or reference material was diluted with Milli-Q water (pH, 6.1; specific conductance, 2 µS cm^{-1}) to prepare a dilution series that ranged from 100% to as low as 5% of the original solute concentration. The pH of the diluted bog water samples ranged from 5.1 (100% bog water) to 5.6 (25% bog water). In one set of experiments conducted at SeaPoint Sensor, Inc., the submerged sensor was subjected to a continuous sequence of four heating–cooling cycles from 5 to 40°C (and back) with data logged at 30-s intervals.

Assessment

Calibration curves for both sensors indicated that CDOM fluorescence intensity gradually curved toward a maximum as the DOM concentration increased (e.g., Fig. 1A). This curvilinear behavior implied that the excitation light could not penetrate deeply enough or that emitted light was resorbed within the sensing volume at high DOM concentrations–or both (i.e., an inner filter effect). Initial experiments also indicated that 1) CDOM fluorescence was negatively related to temperature, 2) a linear function described the relationship well, and 3) the temperature effect was reversible during sequential heating–cooling cycles (Fig. 1B).

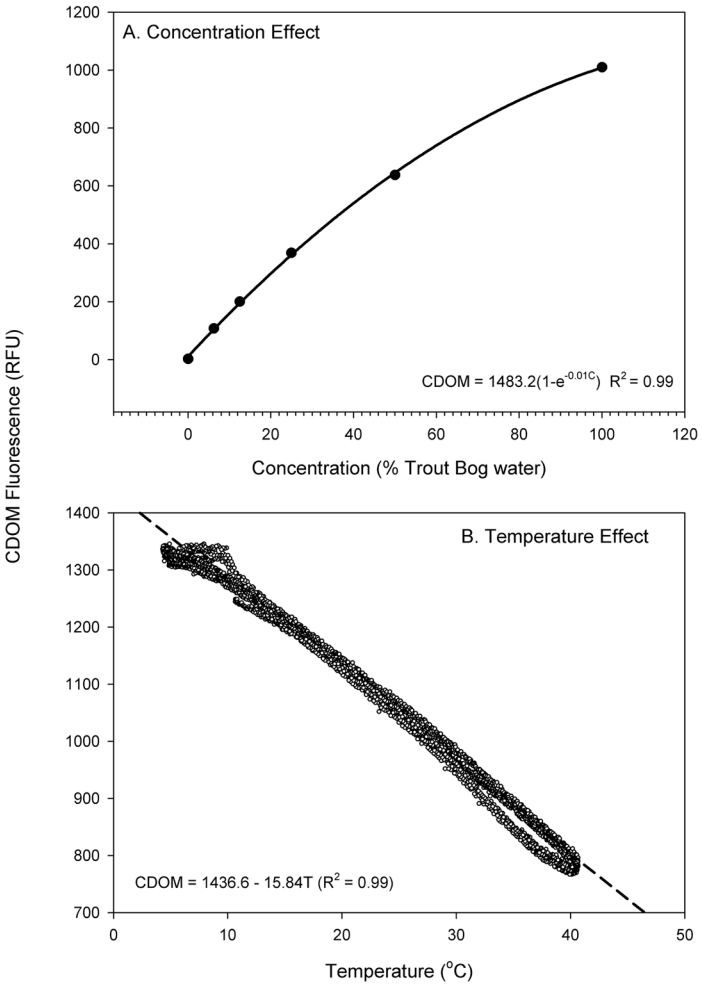

Fig. 1. Effects of DOM concentration (A) and temperature (B) on CDOM fluorescence of TB water in experiments conducted at Seapoint Sensor Inc. during March 2010. Data in (B) indicate some degree of hysteresis during a sequence of four heating–cooling cycles. RFU, relative fluorescence units. DOM concentration at full strength ≈ 17 mg L^{-1}.

Linear regression of data from sequential dilutions of CB and TB water indicated that the slope and intercept of fluorescence intensity as a function of temperature changed proportionately as the OC concentration changed (Figs. 2 & 3). In other words, the ratio slope:intercept was relatively constant regardless of CDOM concentration. This result implied that the following empirical model could be used to compensate for the temperature effect across a wide range of OC concentrations:

$$CDOM_r = CDOM_m/[1 + \rho(T_m - T_r)], \quad (1)$$

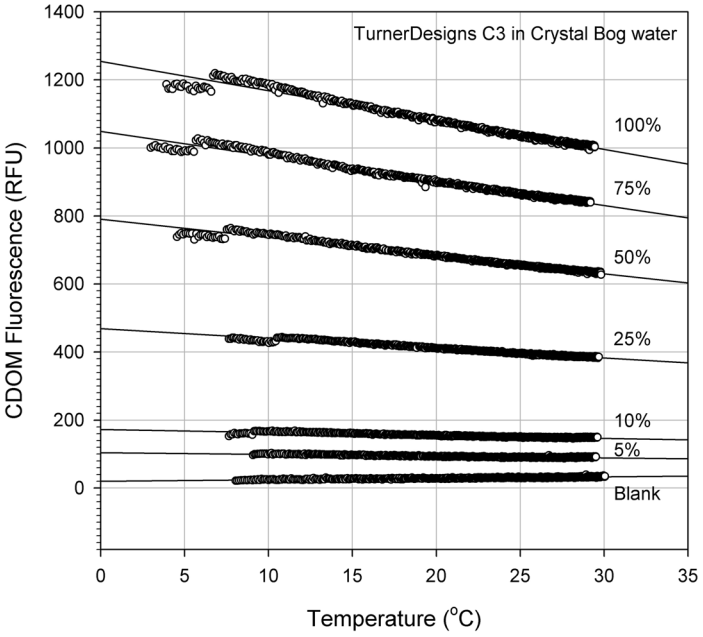

Fig. 2. Effect of temperature on CDOM fluorescence in sequential dilutions of Crystal Bog water (May 2010).

where T is temperature (°C), ρ is the temperature coefficient ($°C^{-1}$), and the subscripts r and m stand for the reference and measured values. In this equation, the temperature coefficient (ρ) is the quotient slope/intercept at a given reference temperature. We note that a general linear regression would have the form: $CDOM_m(c,T_m) = CDOM_r(c) + m(c)(T_m - T_r)$, where c is the concentration (between 0 and 1), $CDOM_r(c)$ is the intercept, and m(c) is the slope. Since the quotient $m(c)/CDOM_r(c)$ is independent of c, we can call this ratio ρ and arrive at the form expressed in Eq. 1.

For the SeaPoint sensor in CB and TB water collected during August, the temperature coefficient, ρ, was estimated to be –0.0155 ± 0.002 (SD) at our chosen reference temperature of 20°C (Fig. 3A&B; Table 1). Using this value for ρ in Eq. 1, the effect of temperature could be removed from the raw lab data for both lakes (Figs. 3C&D). Although CDOM fluorescence declined with temperature for both sensors, the estimated values for ρ differed significantly between sensors (Table 1). Because they were not calibrated to a common standard before experimentation, different offsets may partly explain the differences in ρ. However, differences in optical specifications may also be involved. Absent additional data, we tentatively conclude that ρ is instrument specific.

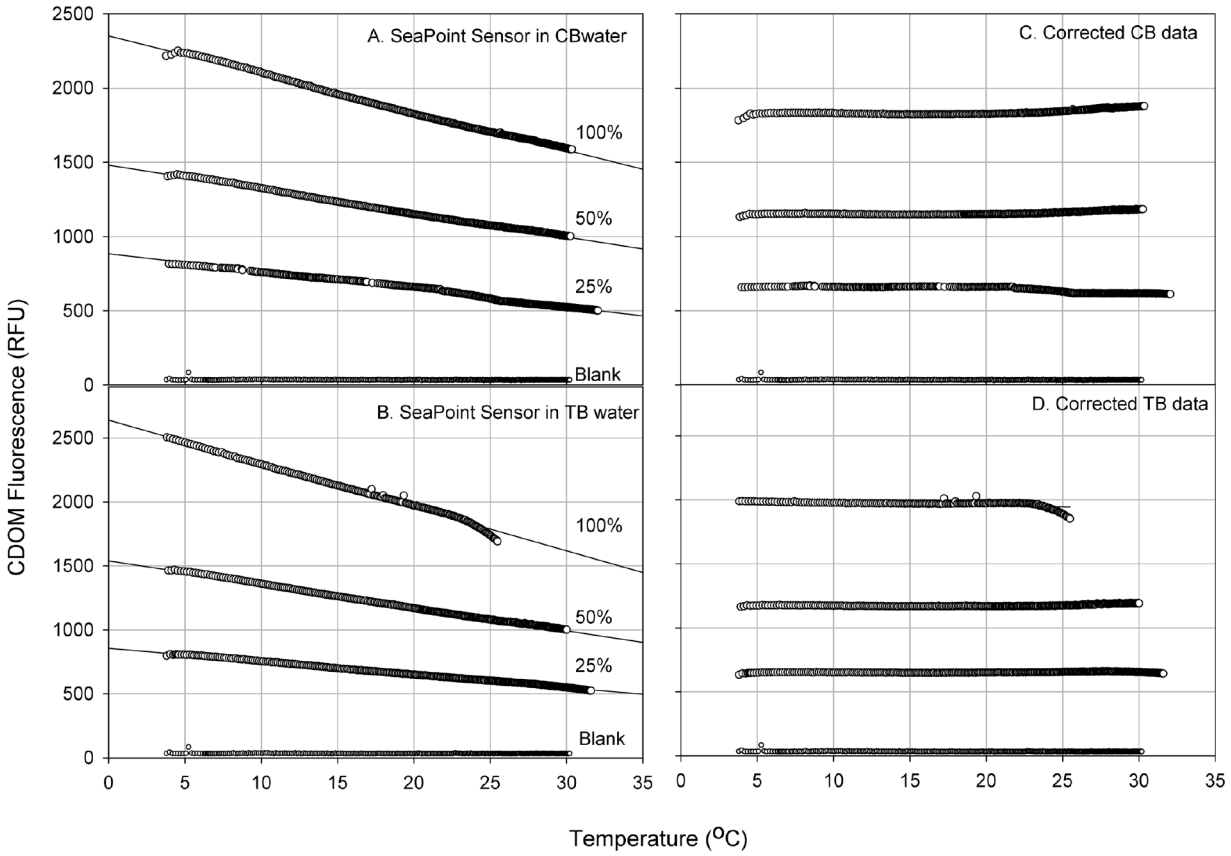

Fig. 3. Effect of temperature on CDOM fluorescence in sequential dilutions of Crystal Bog and Trout Bog water (August 2010). Panels C and D show how the effect of temperature is removed when the raw data are adjusted to a reference temperature of 20°C using the equation $CDOM_{20} = CDOM_m/[1 - \rho(T_m - 20)]$ where $\rho = -0.0155$, the average across all six temperature experiments.

Table 1. Temperature-specific fluorescence coefficients (ρ) for two in situ CDOM fluorometers (reference T = 20°C). CB = Crystal Bog; TB = Trout Bog; Suwannee NOM = IHSS aquatic reference material #1R101N; DOC = 100% concentration; *n* = number of experiments (each experiment conducted at one DOM concentration over the temperature range ~5 to 30°C.)

CDOM Sensor	Matrix	Date	DOC (mg L^{-1})	*n*	Temperature coefficient (ρ) ($°C^{-1}$, mean ±SD)
TurnerDesigns	CB water	May	10.2	6	–0.007 (± 0.001)
	CB water	June	9.8	5	–0.008 (± 0.001)
	CB water	July	11.5	3	–0.009 (± 0.0004)
SeaPoint	CB water	July	11.5	4	–0.015 (± 0.0005)
	CB water	August	12.9	3	–0.015 (± 0.003)
	TB water	August	12.4	3	–0.016 (± 0.0008)
	Suwannee NOM	August	10.3	3	–0.026 (± 0.003)

Experiments with the SeaPoint sensor in CB water, TB water, and reconstituted Suwannee River NOM yielded similar values for ρ (Table 1), even though the OC-source differed. Similarities between CB and TB were not unexpected, given that the dominant OC-source for both is sphagnum-dominated riparian wetland. However, the Suwannee River has a qualitatively different source of OC; and, furthermore, the process of chemical extraction has been shown to modify fluorescence properties (Green and Blough 1994). We caution that the fluorescence behavior of SR NOM was nonlinear at the upper end of the temperature range in our experiments, and those data were not included in the regressions used to estimate ρ on Table 1.

Experiments with quinine sulfate, a common CDOM fluorescence standard, suggested that the effect of temperature was functionally different than that observed with CDOM from natural aquatic sources. With quinine sulfate, fluorescence declined exponentially with temperature at all concentrations tested (Fig. 4B). Consequently, in these experiments, the data can be fitted with a functional relationship: $CDOM_m = CDOM_r\, e^{\rho(Tm-Tr)}$, with decay constant ρ = –0.04 at all four concentrations. We note that this form may further justify the simpler linear relationship that we have presented above, because, if the temperature range is not too large, it can be approximated with $CDOM_m \approx CDOM_r[1 + \rho(T_m-T_r)]$.

The effect of temperature compensation using Eq. 1 on field data are illustrated on Fig. 5. In the raw data, CDOM fluorescence was negatively related to water temperature over daily and weekly time scales (Fig. 5A). This pattern is consistent with our laboratory experiments, and it seems to imply that water temperature can account for the variability in CDOM fluorescence at both time scales. As expected, the effect of a gradual cooling trend was removed after temperature-correction, thereby eliminating an apparent upward trend in CDOM (Fig. 5B). However, the diel cycle of CDOM fluorescence was not removed from the temperature corrected data. Instead, temperature correction damped the diel cycle by flattening daytime values and by decreasing the rate of overnight increase (Fig. 5C). This result implies that a hidden correlate(s) of water temperature was driving the daily CDOM oscillation.

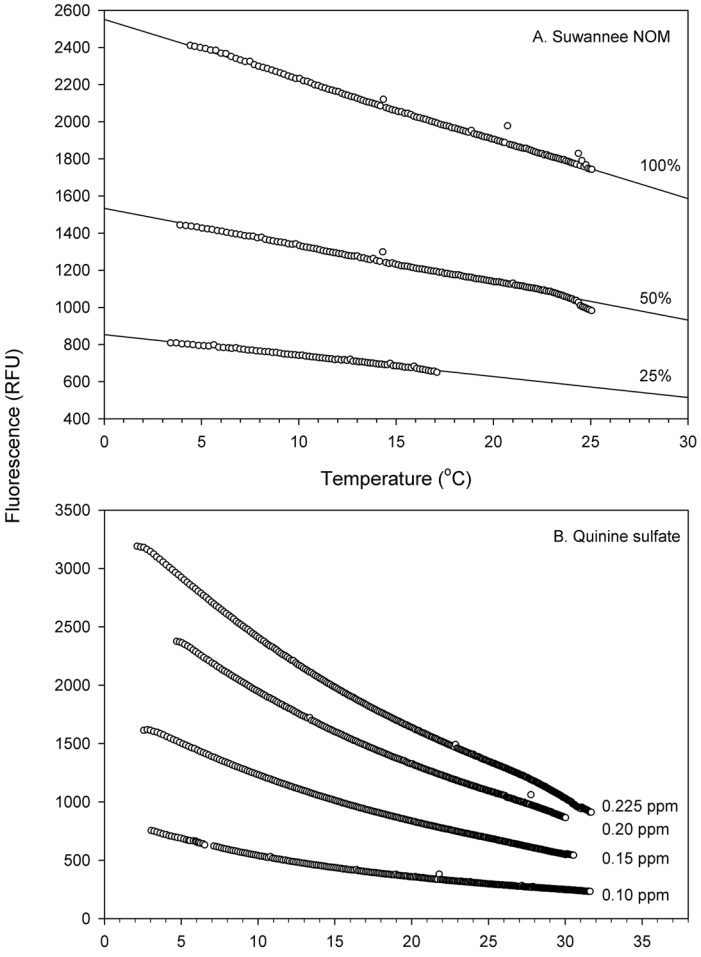

Fig. 4. Effect of temperature on the fluorescence of two commercial reference materials at different concentrations (SeaPoint UV sensor). (A) Suwanee River NOM (IHSS). (B) Quinine sulfate solution.

Discussion

Although the purpose of this methodological study was not to assess the effect of environmental variables other than temperature, ancillary field data indicated that the diel CDOM cycle was not an artifact of ambient sunlight on the fluorom-

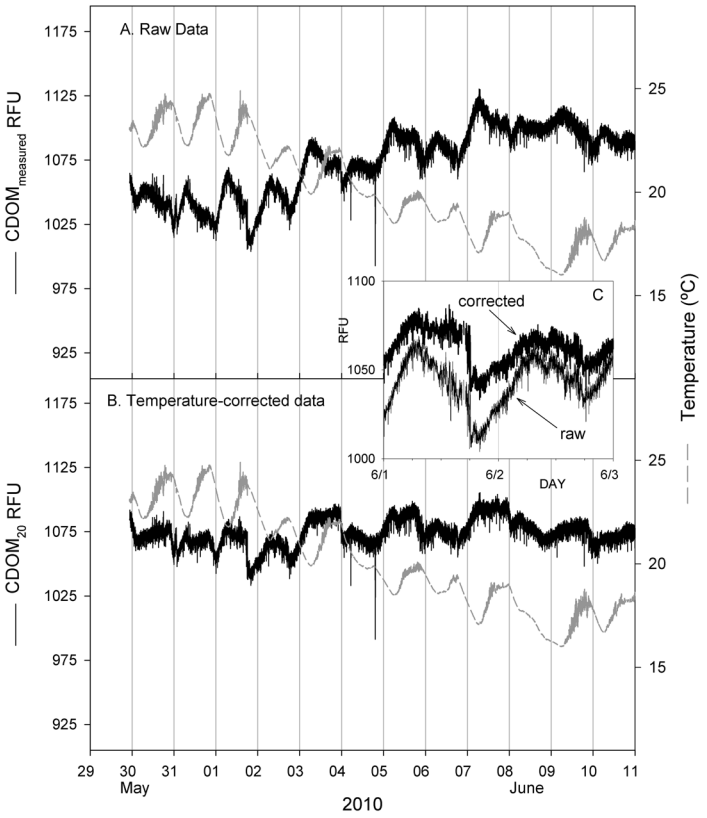

Fig. 5. Comparison of raw (A) and temperature-corrected (B) field data from Crystal Bog using the TurnerDesigns C3 sensor. Solid black line = CDOM fluorescence intensity; broken gray line = water temperature. Insert C shows effect of temperature compensation on the diel CDOM cycle over 2 d (raw data from A; corrected data from B).

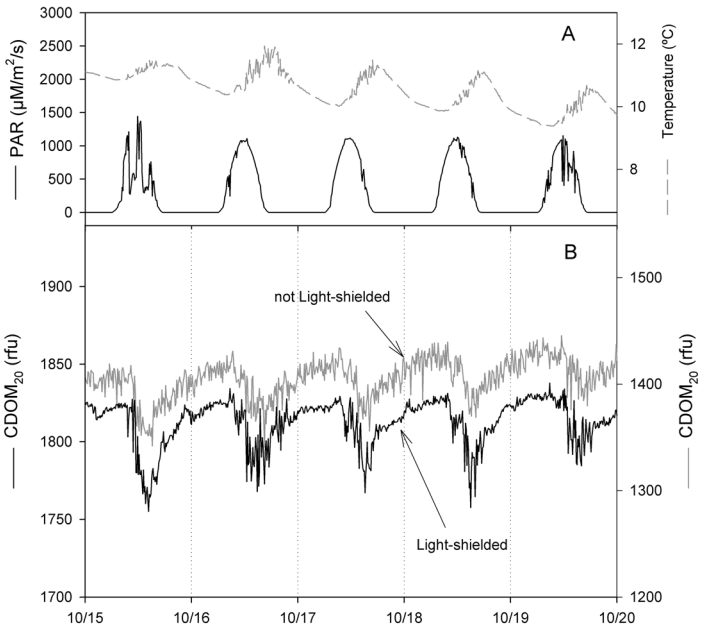

Fig. 6. Effect of the ambient light cycle on the diel CDOM cycle in Crystal Bog during October 2010. Panel A: incident sunlight intensity at the surface and ambient water temperature at the depth of deployment. Panel B: CDOM fluorescence intensity measured simultaneously using in situ fluorometers with (Seapoint sensor) or without (Turner C3 sensor) a light-shielding flow cap.

eters. To test this hypothesis, we placed a light-shielding flow-through cap on the SeaPoint sensor, and then we deployed it alongside the unshielded C3 sensor in Crystal Bog. Water was pumped through the flow-cap using a SeaBird submersible mini-pump. As shown on Fig. 6, the daily CDOM cycle persisted with the light-shield in place; and the diel oscillations were similar between the shielded and unshielded fluorometers.

Daily oscillations in CDOM have been reported in several prior freshwater studies using in situ fluorescence sensors (Spencer et al 2007; Prairie et al. 2010; Sandford et al. 2010). All of these studies reported pronounced diel CDOM cycles similar to what we observed in the uncorrected field data for CB. We suspect that if these prior data were temperature corrected, the diel cycles would be modulated; and, if so, mechanistic explanations might change.

Comments and recommendations

We conclude that temperature compensation is a necessary and important aspect of CDOM monitoring using in situ fluorescence sensors. We propose a method that is analogous to the temperature-compensation method commonly used by limnologists and oceanographers to calculate specific conductance from measurements of electrical conductivity in natural waters. For convenience, we chose 20°C as the reference temperature, and we refer to the corrected data as $CDOM_{20}$.

Temperature-specific coefficients of fluorescence (ρ) were estimated empirically by linear regression of CDOM fluorescence intensity against temperature over the range 5 to 30°C. Although linear equations described our laboratory data well, there was some evidence of nonlinear behavior at temperature extremes. A physical explanation for this behavior is not available because the physics of CDOM fluorescence was beyond the scope of our study.

We conducted multiple experiments to determine the temperature effect across wide DOM concentration gradients. However, because ρ varied independently of DOM concentration, a single DOM concentration should suffice for future determinations with different fluorometers or waters. It remains unclear whether this approach will be valid for marine waters; but we note that Patsayeva et al. (2004) proposed a similar temperature-compensation function for use in remote sensing applications, based on a single experiment with artificial seawater.

Temperature-compensated CDOM fluorescence can be converted to units of DOM (e.g., mg C L^{-1}), given a functional relationship between CDOM fluorescence and DOM concentration at the reference temperature. For data shown on Fig. 1A, a good fit was obtained using

$$CDOM = a(1\text{-}e^{-bC}), \quad (2)$$

where C is the DOM concentration, and a and b are fitted constants. Solving Eq. 2 for C in terms of $CDOM_{20}$ yields:

$$DOM = -\ln (1 - CDOM_{20}/a)/b \quad (3)$$

Methodologically, we recommend a two-step protocol prior to deploying CDOM fluorescence sensors in the field:

1. Determine the temperature-specific fluorescence coefficient (ρ) for the fluorometer by regressing CDOM fluorescence intensity against temperature at an appropriate DOM concentration (cf. Fig. 1B);
2. Calibrate the fluorometer by regressing CDOM fluorescence intensity against DOM concentration at the reference temperature chosen for ρ (cf. Fig. 1A).

After making these preliminary determinations in the laboratory, raw field data can then be corrected by applying Eq. 1 and, if needed, the DOM calibration curve (e.g., Eq. 3). For long field deployments or in very dynamic environments, it may be necessary to repeat these steps to correct for large changes in DOM quality or quantity.

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