

# Comparing the diel cycles of dissolved organic matter fluorescence in a clear-water and two dark-water Wisconsin lakes: potential insights into lake metabolism

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**Abstract:** The cycling of organic carbon is fundamental to aquatic ecosystems, reflecting processes that extend from terrestrial watersheds to fish. Here, we use embedded fluorescence sensors that sample at high frequency to investigate the daily dynamics of a proxy for the major pool of organic carbon (chromophoric dissolved organic matter, CDOM) in a clear-water Wisconsin lake ( $\sim 3 \text{ mg C}\cdot\text{L}^{-1}$ ). We compare the diel CDOM cycle in this lake with cycles observed previously in two dark-water lakes (10 to  $20 \text{ mg C}\cdot\text{L}^{-1}$ ). Despite differences in DOM quality and quantity, diel fluorescence cycles were evident in the epilimnia and hypolimnia of all three lakes. The amplitude differed among lakes, but the timing of the diel cycles was similar, with increases in fluorescence during nighttime and decreases during daylight (except in the aphotic hypolimnion of the darkest lake). The amplitude of the diel cycle increased with increasing DOM concentration, and estimates of DOM turnover based on the magnitude of oscillation ranged from  $0.28 \text{ mg C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$  in the darkest lake to  $0.14 \text{ mg C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$  in the clear lake. Independent estimates of free water metabolism based on the daily dynamics of  $\text{O}_2$  or  $\text{CO}_2$  were in general agreement, ranging from  $0.32$  to  $0.06 \text{ mg C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ . Although absolute rates of turnover varied directly with DOM concentration, relative rates were highest in clear waters ( $\sim 5\%\cdot\text{day}^{-1}$ ). We conclude that these daily oscillations may be a common property of lakes and that they may provide insights into internal DOM processing over short time scales.

**Résumé :** Le cycle du carbone organique est un aspect fondamental des écosystèmes aquatiques, reflétant des processus qui vont des bassins versants terrestres aux poissons. Nous utilisons des capteurs de fluorescence intégrés qui échantillonnent à haute fréquence pour étudier la dynamique journalière d'une variable substitutive du grand réservoir de carbone organique (la matière organique dissoute chromophorique, MODC) dans un lac d'eau claire du Wisconsin ( $\sim 3 \text{ mg C}\cdot\text{L}^{-1}$ ). Nous comparons le cycle journalier de la MODC dans ce lac aux cycles observés antérieurement dans deux lacs d'eau sombre (de 10 à  $20 \text{ mg C}\cdot\text{L}^{-1}$ ). Malgré des différences sur le plan de la qualité et de la quantité de la MOD, des cycles de fluorescence journaliers étaient évidents dans l'épilimnion et l'hypolimnion des trois lacs. L'amplitude variait selon le lac, mais la séquence des cycles quotidiens était semblable, comprenant des augmentations de la fluorescence durant la nuit et des diminutions durant le jour (sauf dans l'hypolimnion aphotique du lac le plus sombre). L'amplitude du cycle journalier augmentait parallèlement à la concentration de MOD, et les estimations du brassage de la MOD reposant sur la magnitude des oscillations allaient de  $0,28 \text{ mg C}\cdot\text{L}^{-1}\cdot\text{jour}^{-1}$  dans le lac le plus sombre à  $0,14 \text{ mg C}\cdot\text{L}^{-1}\cdot\text{jour}^{-1}$  dans le lac clair. Des estimations indépendantes du métabolisme de l'eau libre reposant sur la dynamique journalière de l' $\text{O}_2$  ou du  $\text{CO}_2$  concordaient en général, allant de  $0,32$  à  $0,06 \text{ mg C}\cdot\text{L}^{-1}\cdot\text{jour}^{-1}$ . Même si les taux absolus de brassage étaient directement corrélés à la concentration de MOD, les taux relatifs étaient les plus élevés dans les eaux claires ( $\sim 5\%\cdot\text{jour}^{-1}$ ). Nous concluons que ces oscillations journalières pourraient être une propriété répandue des lacs et qu'elles pourraient contribuer à une meilleure compréhension du traitement interne de la MOD à de courtes échelles temporelles. [Traduit par la Rédaction]

## Introduction

Dissolved organic matter (DOM) is an important constituent of freshwater ecosystems, often exceeding the mass of carbon in live organisms and detritus in the water column (Wetzel 2001). DOM in fresh waters originates from either allochthonous sources such as terrestrial soils and plant material or autochthonous sources such as plankton exudates and the decomposition of macrophytes (Thurman 1985; Wetzel 2001; Tranvik et al. 2009). While the relative contribution of these DOM sources varies across the landscape, recent research indicates that the DOM pool of lakes in northern Wisconsin, USA, is composed almost entirely of terrigenous

matter (based on stable isotope studies; Wilkinson et al. 2013a). Further, understanding the dynamics of DOM in aquatic ecosystems is critical given its role in the aquatic carbon cycle (Buffam et al. 2011), as a source of organic acids (Buffam et al. 2007), light penetration and UV absorption (Morris et al. 1995), metal binding (Reuter and Perdue 1977), and carbon source for microbial communities and higher trophic levels (Karlsson et al. 2012; Wilkinson et al. 2013b).

Traditional assays of DOM concentration in fresh waters are time-consuming and expensive, but the development of in situ sensors that measure the fluorescence of chromophoric DOM

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**Table 1.** Limnological characteristics of clear-water Sparkling Lake compared with the two dystrophic lakes (North Temperate Lakes Long-Term Ecological Research program).

	Sparkling Lake	Trout Bog	Crystal Bog
Latitude	46.008	46.041	46.008
Longitude	-89.701	-89.686	-89.606
Surface area (ha)	63.7	1	0.6
Depth (m)	20	7.9	2.5
Catchment area (ha)	140	14	4.8
pH	7.4	4.8	5.2
ANC ( $\mu\text{equiv}\cdot\text{L}^{-1}$ )	631	11	14
DOC ( $\text{mg}\cdot\text{L}^{-1}$ )	3.4	19.9	10.6
Conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ )	86	23	11
TN ( $\mu\text{g}\cdot\text{L}^{-1}$ )	371	961	722
TP ( $\mu\text{g}\cdot\text{L}^{-1}$ )	15	46.6	18.2
$S_{275-295}$ ( $\text{nm}^{-1}$ ) <sup>a</sup>	0.0266 (0.0035, 19)	0.0139 (0.0003, 20)	0.0135 (0.0003, 20)

Note: ANC, acid neutralizing capacity; DOC, dissolved organic carbon; TN, total nitrogen; TP, total phosphorus.

<sup>a</sup>Spectral slopes ( $S_{275-295}$ ) were estimated for quarterly surface samples, 2010–2014; data shown are mean (SD,  $n$ ). See Helms et al. (2008).

(CDOM fluorescence or FDOM) has enabled high-frequency measurements that open promising lines of research on the carbon cycle and lake metabolism (Coble et al. 2014). Using CDOM fluorescence as a DOM proxy in fresh waters, several investigators have reported diel cycles attributable to such factors as the daily entrainment of hypolimnetic waters (Gibson et al. 2001), a combination of photochemical and biologically mediated processes (Spencer et al. 2007; Saraceno et al. 2009), and the daily cycling of external hydrologic loads (Pellerin et al. 2012). Potential interactions among multiple processes can complicate interpretations of CDOM fluorescence patterns.

In a recent study of CDOM fluorescence in two dystrophic Wisconsin lakes, we reported diel cycles that were not readily attributable to factors such as instrumental artifact, hydrologic forcing, solar radiation, inner filtering, pH, or redox conditions (Watras et al. 2015). Instead, the daily dynamics in these high-humic lakes were apparently governed by biological processes that mediate DOM production (release) and destruction (uptake). This hypothesis was supported by similarities between rates of carbon turnover derived from properties of the diel fluorescence cycle ( $0.28 \text{ mg C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ ) and rates of net ecosystem production (NEP) based on daily  $\text{CO}_2$  dynamics ( $0.32 \text{ mg C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ ).

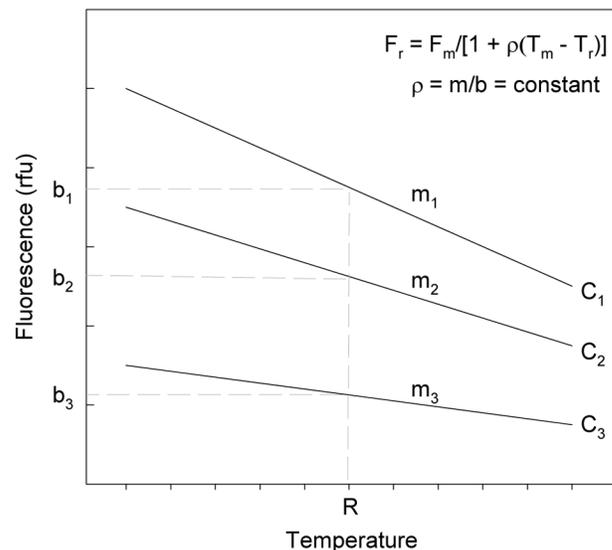
Because photobleaching and photomineralization are known to have profound effects on the chemical structure, optical properties, and bioavailability of aquatic DOM (Sulzberger and Durisch-Kaiser 2009), and because solar exposure is relatively low in the water column of high-humic waters (especially in the UV region, Scully and Lean 1994; Molot and Dillon 1997), we now investigate the dynamics of CDOM fluorescence in a clear-water lake where a larger proportion of the water column is exposed to solar irradiation. We compare fluorescence patterns in the clear-water lake with those previously observed in the two dystrophic lakes, focusing on diel oscillations and their potential importance. To minimize the effect of variable hydrologic loads, we confine the comparison to seepage lakes that have no fluvial sources of DOM. We compare CDOM fluorescence patterns in oxic surface waters and anoxic hypolimnetic waters, and we explore implications with respect to lake metabolism.

## Methods

### Study sites

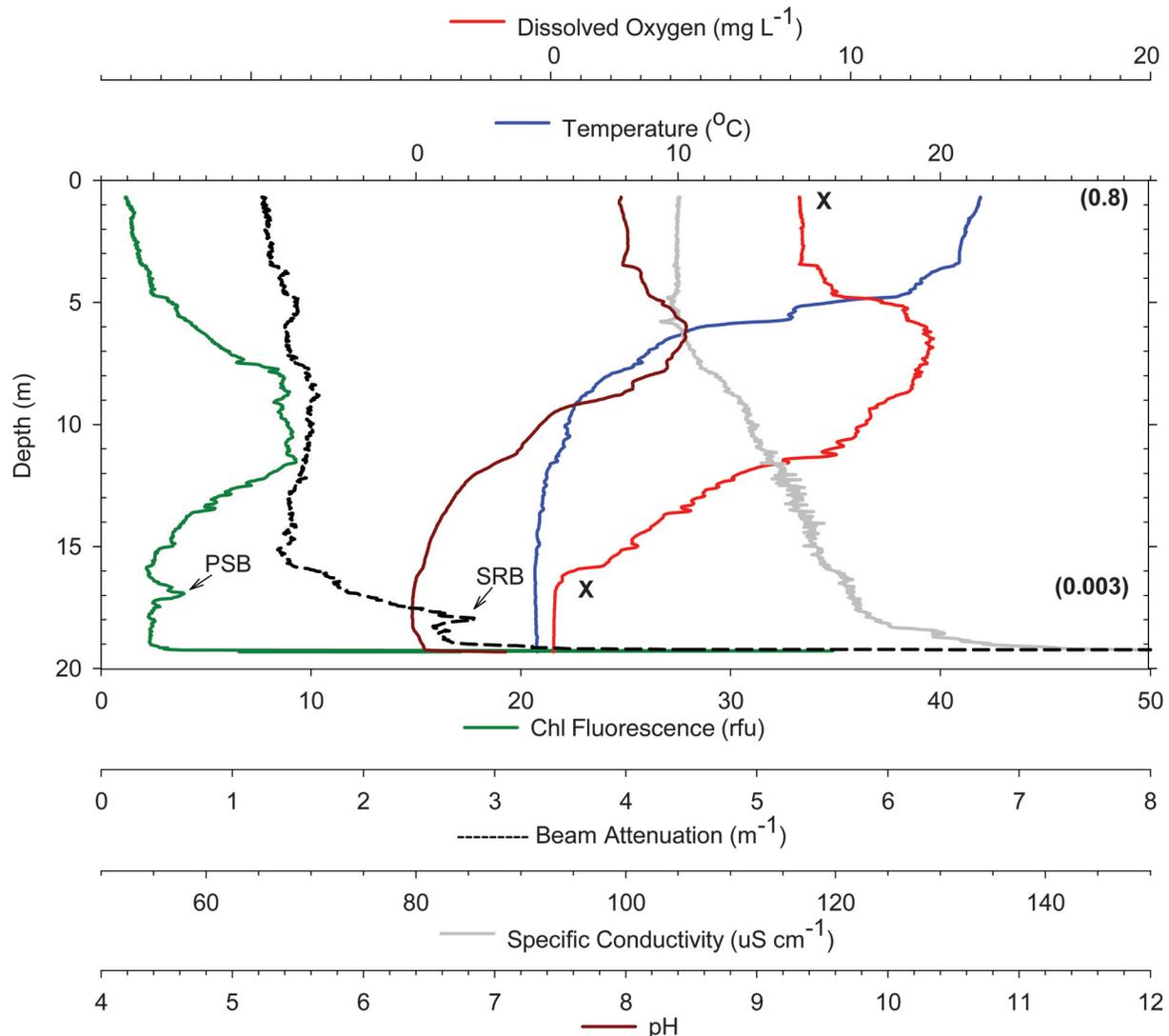
The clear-water study site was Sparkling Lake, a seepage lake situated in forested uplands composed of outwash sand and deep glacial till in northern Wisconsin (Attig 1984). Located about 2 km west of the Trout Lake research station, it is one of several lakes in the Northern Temperate Lakes Long-Term Ecological Research

**Fig. 1.** Linear temperature compensation model for chromophoric dissolved organic matter (CDOM) fluorescence in fresh waters ( $F$  = fluorescence,  $T$  = temperature, subscripts  $m$  and  $r$  are measured and reference, respectively). Plot shows a family of curves for a dilution series of lake water (three concentrations,  $C_i$ ).  $R$  is the chosen reference temperature,  $m_i$  are the slopes at each concentration, and  $b_i$  are the intercepts at the reference temperature. The reference temperature can be chosen arbitrarily. The temperature-specific coefficient ( $\rho$ ) is constant over all concentrations, and it is estimated as the quotient of the slope ( $m$ ) divided by the intercept at the reference temperature ( $b$ ). Note that  $\rho$  has the units ( $^{\circ}\text{C}^{-1}$ ) for dimensional consistency. Because this is a linear model,  $\rho$  cannot be expressed as  $\% \cdot ^{\circ}\text{C}^{-1}$ . See Watras et al. (2011) for the empirical derivation.



program (NTL-LTER, [www.limnology.wisc.edu](http://www.limnology.wisc.edu)) and the Global Lake Ecological Observatory Network (GLEON, [www.gleon.org](http://www.gleon.org)). The dynamics of CDOM fluorescence in Sparkling Lake are compared with those reported previously for two nearby dystrophic lakes (Crystal Bog and Trout Bog) that are situated in *Sphagnum*-dominated subcatchments of the Trout Lake watershed (Watras et al. 2015). Since none of the three study lakes has inflowing or outflowing streams, hydrologic inputs are limited to direct precipitation and subsurface discharge.

**Fig. 2.** Bio-optical profile for Sparkling Lake during midsummer 2014. An X marks depth of CDOM sensors. Numbers in parentheses are the fraction of incident solar radiation (PAR) reaching the depth of each CDOM sensor. PSB, presumptive layer of photosynthetic sulfur bacteria; SRB, presumptive layer of sulfate-reducing bacteria.



Limnological characteristics of the three lakes are compared in Table 1. Because of seepage from surrounding peatlands, terrigenous DOC of wetland origin is the dominant solute in the two bog lakes (Hanson et al. 2014). Since Sparkling Lake has no contiguous peatland, DOC concentrations are substantially lower and the water is relatively clear. Nonetheless, stable isotope studies indicate that DOM in Sparkling Lake is also almost entirely derived from terrestrial sources (Wilkinson et al. 2013a). Mass balance modeling is consistent with this finding and indicates that the dominant terrestrial sources to Sparkling Lake are aerial deposition and surface runoff (Hanson et al. 2014).

#### Fluorescence measurements

Two submersible CDOM fluorometers were deployed from May to November 2014 in Sparkling Lake: (i) a SeaPoint UV Fluorometer from SeaPoint Sensors was deployed in the epilimnion at a depth of 0.5 m; (ii) a Cyclops-7 Fluorometer from TurnerDesigns was deployed in the hypolimnion at a depth of 16.5 m. Fluorescence specifications for the SeaPoint sensor were as follows: Ex 370 nm center wavelength (CWL), 12 nm full width at half maximum wave height (FWHM); Em 440 nm CWL, 40 nm FWHM. Fluorescence specifications for the TurnerDesigns C7 were as follows: Ex 325 nm CWL,

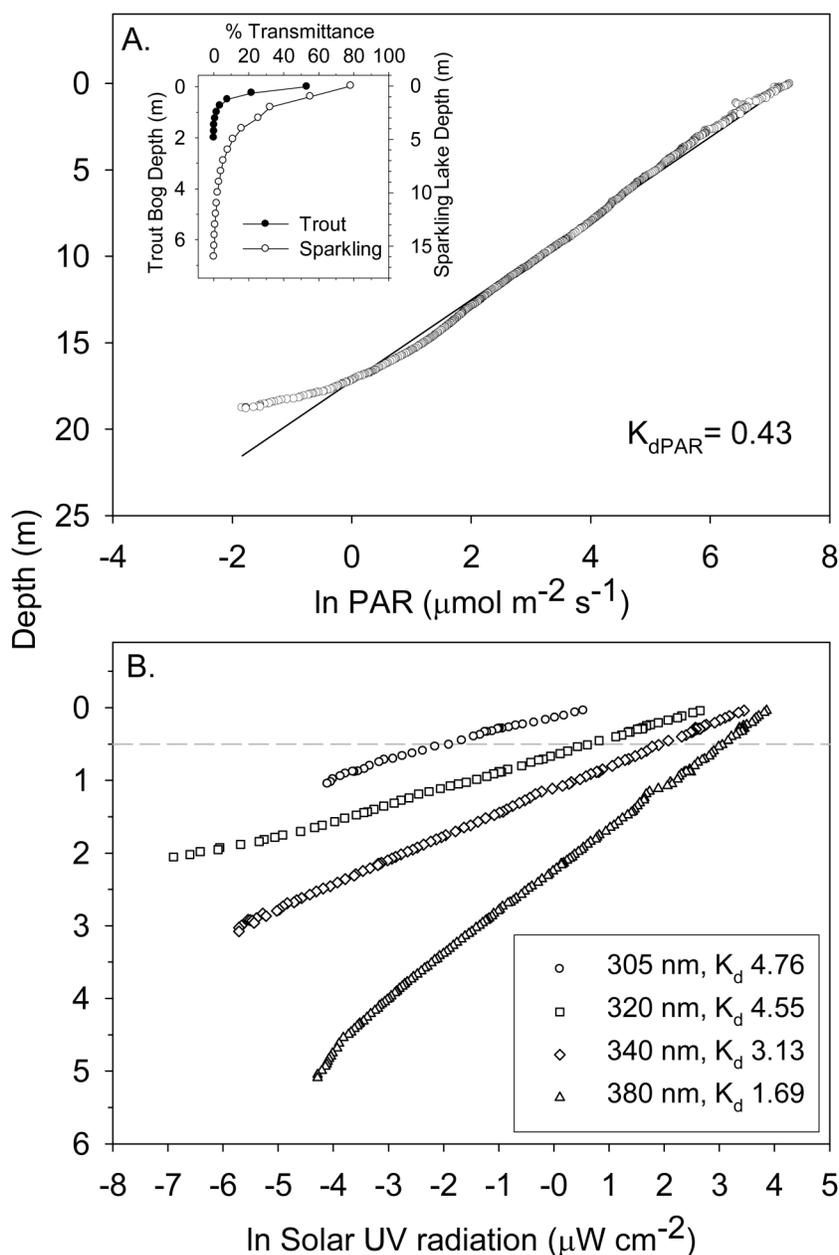
120 nm FWHM; Em 470 nm CWL, 60 nm FWHM. These open-face sensors measure total fluorescence within their sampling volume, potentially including particulates, which we collectively designate by convention as “CDOM” (see Conmy et al. 2014 for alternative nomenclature).

The fluorescence sensors were deployed from instrumented buoys moored near the center of the lake. The buoys provided 12 V DC power, ancillary sensors, data-logging capability, and radio communication. Fluorescence data were logged at 30 min intervals and transmitted to the University of Wisconsin Trout Lake Station via radio twice daily. To prevent signal degradation due to biofouling, the fluorometers were cleaned manually in the field each week. Prior to deployment, output from the two fluorometers was scaled to a common reference sample of lake water. All fluorescence data were corrected for temperature quench and reported as CDOM<sub>20</sub> as described below.

#### Temperature compensation

Thermistors were co-located at the depth of each fluorometer, and water temperature was recorded at 30 min time intervals along with CDOM fluorescence. Raw fluorescence data were cor-

Fig. 3. Solar irradiance profiles for Sparkling Lake under clear-sky conditions during midsummer. Insert in panel A shows comparison with Trout Bog. Solid line in panel A is the linear regression. Dashed line in panel B indicates depth of CDOM sensor in epilimnion.



rected for temperature quench using the linear temperature compensation model

$$(1) \quad \text{CDOM}_r = \text{CDOM}_m / [1 + \rho(T_m - T_r)]$$

where  $T$  is temperature ( $^{\circ}\text{C}$ ),  $\rho$  is the temperature coefficient ( $^{\circ}\text{C}^{-1}$ ), and the subscripts  $r$  and  $m$  stand for the reference and measured values, respectively (Watras et al. 2011). In this model,  $\rho$  is calculated as the quotient of two parameters derived from a linear regression of fluorescence on temperature obtained under laboratory conditions (Fig. 1). We chose a reference temperature of  $20^{\circ}\text{C}$ , and the temperature-corrected data are thus reported as  $\text{CDOM}_{20}$ .

#### Time series analysis

Periodicity in the time series for  $\text{CDOM}_{20}$  was investigated by Fourier spectral analysis. The fast Fourier transform (FFT) decom-

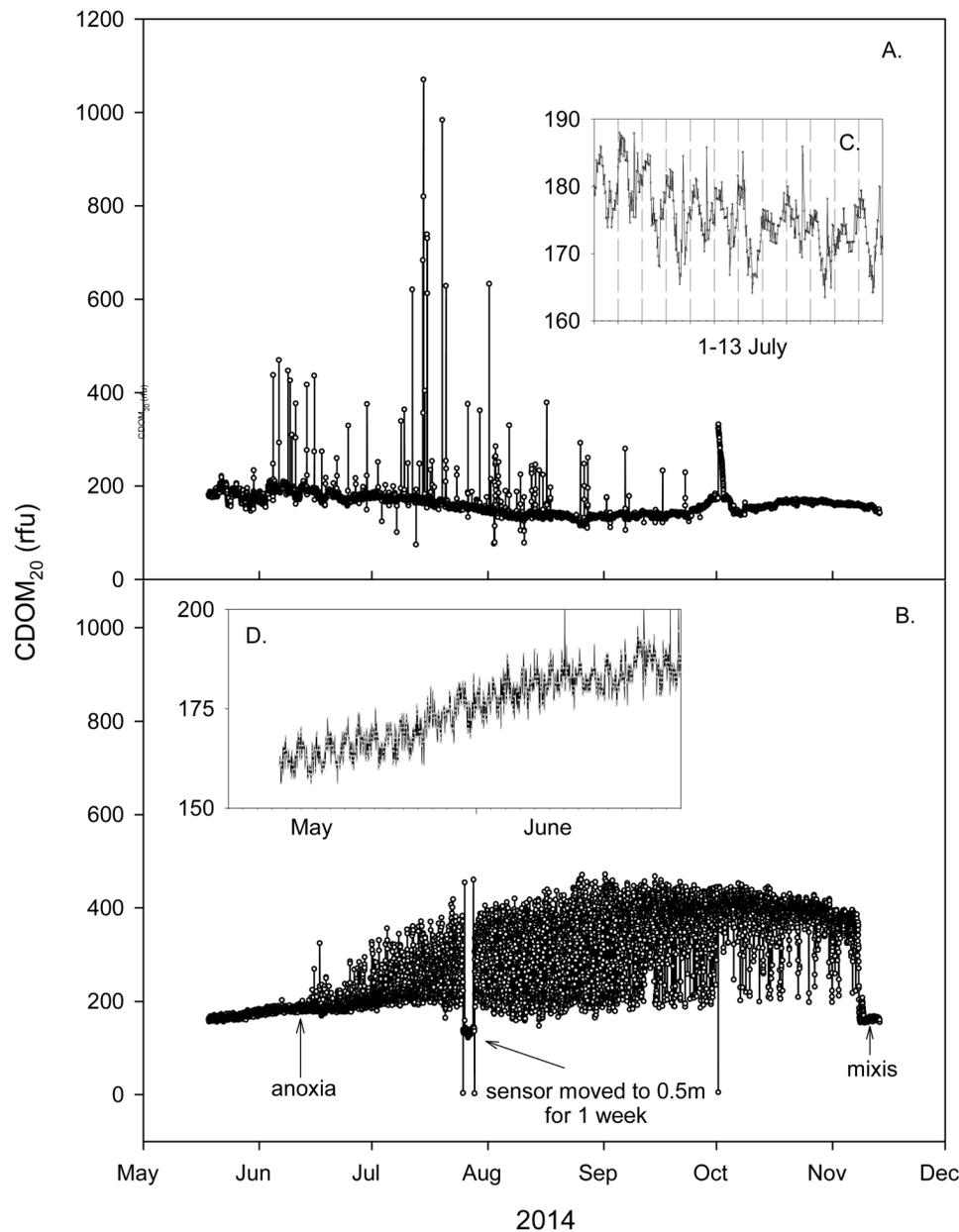
poses a function into a sum of sinusoids with different frequencies, and power spectra can be used to diagnose whether a given frequency stands out with sufficiently high amplitude to suggest significant periodicity. Prior to applying the FFT obvious outliers were culled and the data were detrended by removing the best linear fit over the time span of interest. AutoSignal 1.7 (Systat Software, Inc.) was used for these analyses.

To further investigate frequencies of one cycle per day, we binned the detrended  $\text{CDOM}_{20}$  values into hours of the day (1 to 24) and calculated the mean  $\text{CDOM}_{20}$  value for each hour over the time series of interest. Plotting time of day ( $x$ ) against the mean  $\text{CDOM}_{20}$  value ( $y$ ) provided a direct visual assessment of the amplitude and timing of the diel cycle (or lack of one).

#### Ancillary field measurements

Vertical profiles of specific conductivity, temperature, chlorophyll  $a$  fluorescence, horizontal beam attenuation (i.e., optical density),

**Fig. 4.** Time series for CDOM<sub>20</sub> in (A) the epilimnion (0.5 m) and (B) the hypolimnion (16.5 m) of Sparkling Lake during 2014. Insert C shows a 12-day time segment from panel A with out-of-range values removed. Insert D shows the diel hypolimnetic CDOM cycle during early summer before large spikes dominate the time series. Note that in late July the sensor was moved into the epilimnion for 1 week to determine if the erratic signal in the hypolimnion was due to a problem with sensor performance.



pH, and dissolved oxygen (DO) were obtained for the water column of Sparkling Lake using a sensor package from SeaBird Electronics (Bellevue, Washington). The sensors included a SBE19plus CTD, a SBE43 DO sensor, a SBE18-I pH sensor, a WET Labs WETSTAR chlorophyll fluorescence sensor, and a WET Labs C-Star transmissometer. The sensors were plumbed to twin SBE 5T submersible pumps, which ensured that each sensor measured the same parcel of water during descent. The sensor package was lowered at a rate of about 2 cm·s<sup>-1</sup> using an electric winch. Each sensor sampled at a rate of 4 Hz and reported a single averaged value for each second, yielding roughly 2 cm vertical resolution of the measured variables.

Solar irradiance profiles were obtained in Sparkling Lake using a PUV-2500 multi-channel radiometer (Biospherical Instruments Inc.) that measured photosynthetically active radiation (PAR, 400–

700 nm) and ultraviolet radiation (UV) at four wavelengths (305, 320, 340, and 380 nm). The radiometer was manually lowered through the water column sampling at a frequency of 1 s<sup>-1</sup> until contacting the bottom of the lake.

Additional limnological data (Table 1) were obtained from the NTL-LTER database maintained by the Center for Limnology, University of Wisconsin–Madison ([www.limnology.wisc.edu](http://www.limnology.wisc.edu)).

### Experimental

A laboratory experiment was conducted to assess the potential effect of zooplankton on the CDOM<sub>20</sub> fluorescence signal. Two 6 L Pyrex beakers were filled with 4 L of epilimnetic water from Sparkling Lake (with and without zooplankton). The beakers were placed on a black surface to minimize reflectance. CDOM fluorescence (C7 Cyclops) and temperature were measured for ~10 min

**Table 2.** Estimates of dissolved organic carbon (DOC) turnover based on the magnitude of the daily CDOM<sub>20</sub> (temperature-corrected (20 °C) data for chromophoric dissolved organic matter) oscillation (peak to trough) compared with rates derived from dissolved O<sub>2</sub> or CO<sub>2</sub> daily dynamics.

Lake	Layer	CDOM <sub>20</sub> (rfu)	DOC (mg C·L <sup>-1</sup> )	Calibration (rfu·mg C <sup>-1</sup> ·L <sup>-1</sup> )	Oscillation (rfu·day <sup>-1</sup> )	Turnover (mg C·L <sup>-1</sup> ·day <sup>-1</sup> )	
						ΔCDOM <sub>20</sub> basis	ΔO <sub>2</sub> or ΔCO <sub>2</sub> basis
Sparkling Lake	Epilimnion	158	2.8	56.4	8	0.14	0.06 <sup>a</sup>
	Hypolimnion	182	2.7	67.4	6	0.09	NA
Trout Bog	Epilimnion	1686	17.7	95.2	26.6	0.28	0.32 <sup>b</sup>
	Hypolimnion	2343	23.8	98.4	10.4	0.10	NA
Crystal Bog	Epilimnion	1075	10	107.5	25	0.23	NA

**Note:** Data are mean values for summer 2014. Results for Trout Bog and Crystal Bog gleaned from Watras et al. (2015). NA, not applicable.

<sup>a</sup>Rate derived from dissolved O<sub>2</sub>.

<sup>b</sup>Rate derived from dissolved CO<sub>2</sub>.

trials sampling at a rate of 10 Hz. The zooplankton were collected from Sparkling Lake using a 30 cm diameter Wisconsin net (163 μm Nitex mesh) hauled vertically from 10 m depth to the surface. Just prior to experimentation, live zooplankton were re-concentrated using a 7 cm plastic ringnet (150 μm Nitex mesh) and backwashed into one of the beakers. The zooplankton assemblage was dominated by adult *Daphnia*, cyclopoid copepods, and diaptomid copepods.

**DO**

To independently estimate rates of free-water metabolism, high-frequency measurements of DO were made in the epilimnion of Sparkling Lake using an optical probe suspended from the instrumented lake buoy (Zebra-Tech, D-Opto). DO, water temperature, and meteorological data were collected continuously from the buoy at 1 min time intervals throughout the ice-free season and reported via radio to the Trout Lake Station along with the CDOM fluorescence measurements. The optical DO probe was corrected for drift each week using a calibrated hand-held sensor (YSI Pro-ODO).

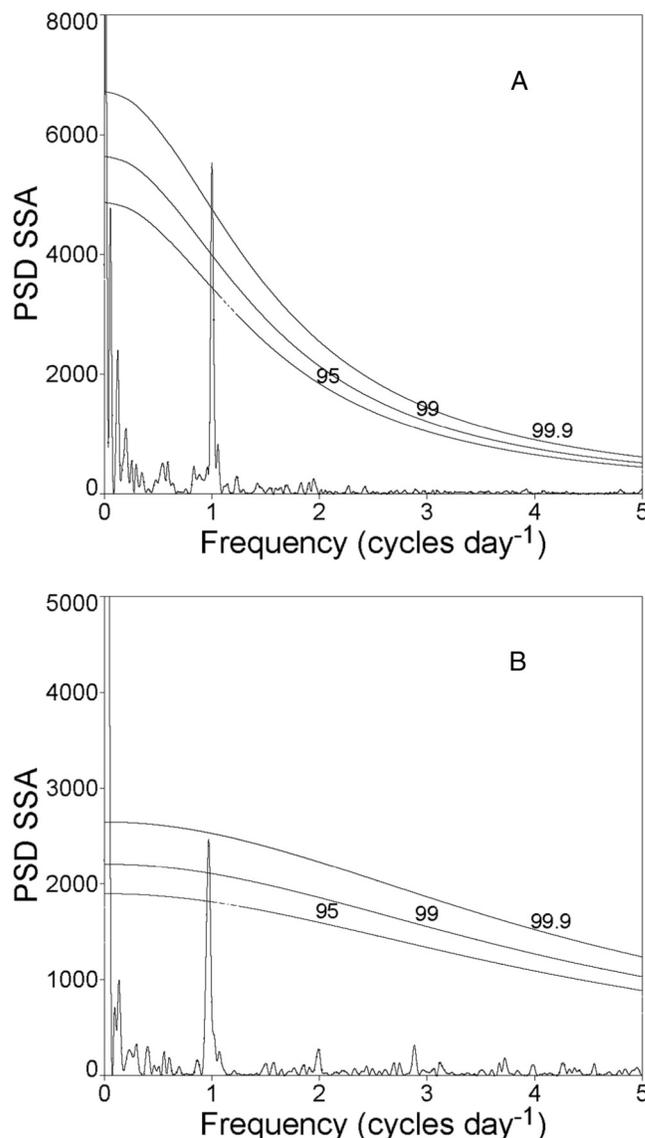
**Results and discussion**

**Physical structure of the water column**

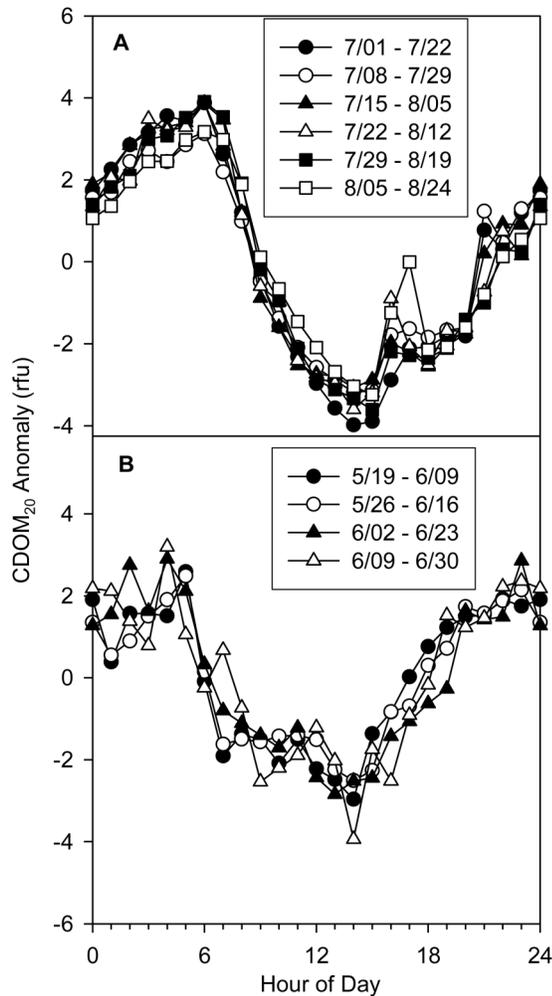
Sparkling Lake stratified strongly during summer, with a broad metalimnetic oxygen maximum (associated with elevated chlorophyll and pH) above a narrow anoxic hypolimnion (Fig. 2). In the hypolimnion, there was evidence that a microstratified assemblage of anaerobic microbes occupied the region of the deep CDOM sensor just below the oxic-anoxic (O-A) boundary (Fig. 2), presumably photosynthetic sulfur bacteria and (or) sulfate reducers as reported by Vila et al. (1998). The vertical structure of the Sparkling Lake water column was in marked contrast with the structure of the bogs. Trout Bog has a shallow epilimnion (~1 m) over a large anoxic hypolimnion (~7 m), and Crystal Bog is polymictic (Watras et al. 2015).

Solar irradiance profiles indicate that light attenuation was roughly an order of magnitude higher in Trout Bog than in Sparkling Lake ( $K_{dPAR}$  3.4 versus 0.43; Fig. 3A). In Sparkling Lake, 70% to 80% of the incident PAR reached the depth of the epilimnetic CDOM sensor compared with ~8% in Trout Bog (Fig. 3A insert). PAR was low but still measurable in the Sparkling Lake hypolimnion, whereas it was below detection in the hypolimnion of Trout Bog (Watras et al. 2015). More importantly, the extinction of highly photolytic UV radiation was extremely high in Trout Bog ( $K_{dUV} \approx 25 \text{ m}^{-1}$ ; Watras et al. 2015), while in Sparkling Lake, the  $K_{dUV}$  ranged from 1.7 to 4.8  $\text{m}^{-1}$  depending on wavelength, and there was measurable UVA and UVB at the depth of the epilimnetic sensor (Fig. 3B). These results indicate relatively high solar irradiation levels in the Sparkling Lake epilimnion and the exist-

**Fig. 5.** Spectral analysis of time series for CDOM<sub>20</sub> in Sparkling Lake (fast Fourier transform, FFT): (A) sensor at 0.5 m; 1 July – 24 August 2014; (B) sensor at 16.5 m, 18 May – 30 June 2014. All data detrended; cs2Hann window; PSD SSA, power spectral density as sum squared amplitude. Lines indicate significance levels (red noise model).



**Fig. 6.** Diel cycle of CDOM<sub>20</sub> anomalies in Sparkling Lake plotted using hourly binned data. Data were blocked into sequential 3-week segments (with overlap) to minimize variability due to nonstationarity in the full time series. (A) Epilimnetic sensor at 0.5 m depth. (B) Hypolimnetic sensor at 16.5 m depth. Extreme values were removed before calculating the mean value for each hour of the day.



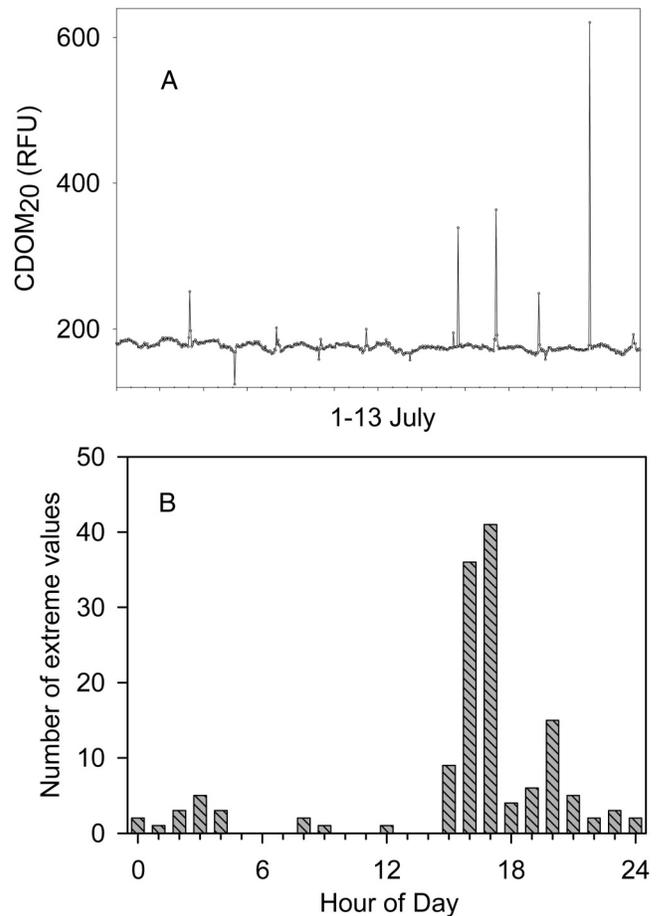
tence of a diel solar cue in the hypolimnion, both of which were lacking in dystrophic Trout Bog.

**Patterns of CDOM fluorescence**

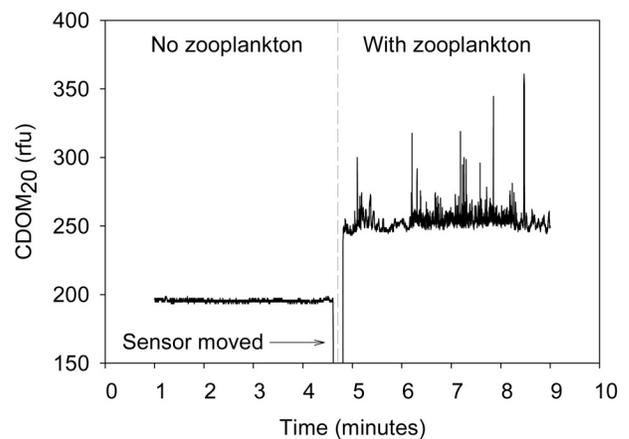
The CDOM<sub>20</sub> fluorescence signal in Sparkling Lake was roughly an order of magnitude lower than that observed in the bogs, averaging  $162 \pm 32$  (mean  $\pm$  SD) relative fluorescence units (rfu) in the epilimnion and  $184 \pm 96$  rfu in the hypolimnion over the time period from May to November 2014 (Fig. 4; Table 2). The epilimnetic time series was relatively stationary from spring through fall (Fig. 4A), but in the hypolimnion there was a gradual increase in fluorescence until 8 November when it equilibrated with the epilimnion (160 rfu) during mixis (Fig. 4B).

Two features dominated both fluorescence time series in Sparkling Lake. The first was the diel cycle, which had an amplitude of about 4 rfu in the epilimnion and 3 rfu in the hypolimnion (Figs. 4C, 4D). Spectral analysis confirmed a dominant periodicity of 1 cycle-day<sup>-1</sup> in both time series (Fig. 5). Graphical analysis of the hourly binned data indicated that fluorescence decreased during the day and increased during the night at both depths, reaching peak values near sunrise (Fig. 6). This pattern is similar to that

**Fig. 7.** (A) Time series from Fig. 4C showing daily spikes in fluorescence superimposed on the diel CDOM<sub>20</sub> cycle. (B) Frequency histogram of fluorescence spikes from time series for CDOM<sub>20</sub> from 1 July to 24 August 2014 (cf. Fig. 4A).

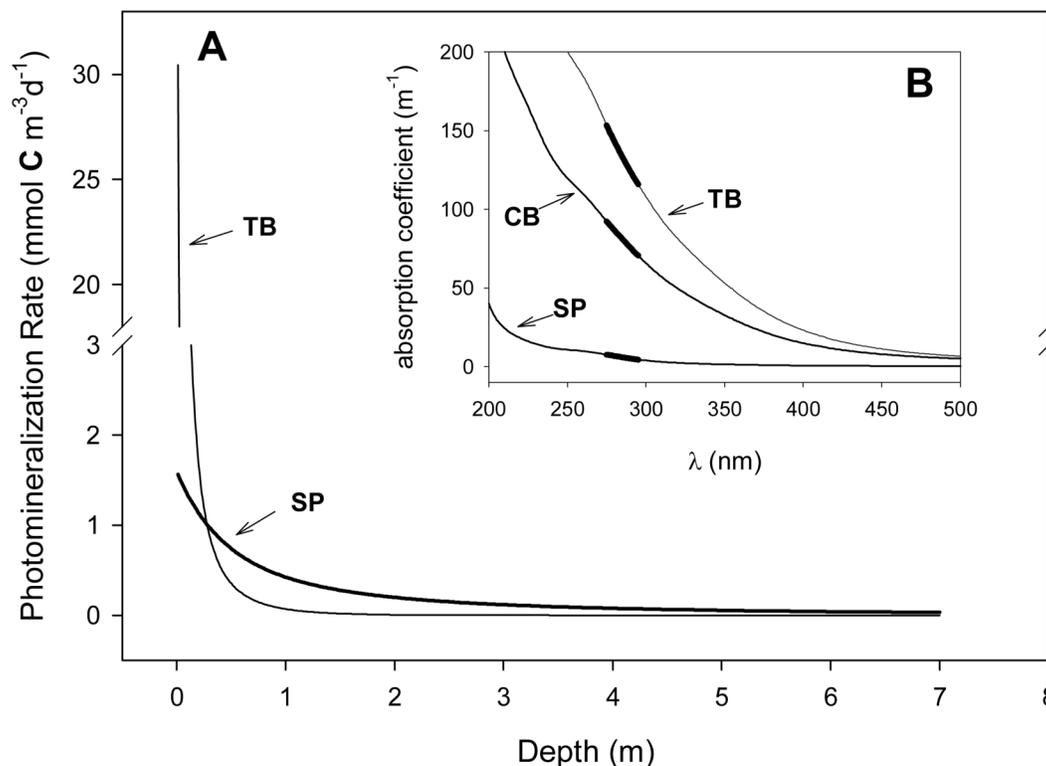


**Fig. 8.** Effect of crustacean zooplankton (cladocera and copepods) on CDOM<sub>20</sub> fluorescence under laboratory conditions.



observed in the epilimnia of the bogs (Watras et al. 2015), although the daily amplitude in Sparkling Lake was substantially lower than that in the bogs (approximately threefold). In addition, the epilimnetic and hypolimnetic cycles in Trout Bog were asynchronous (the hypolimnetic cycle peaked around solar noon rather than sunrise; Watras et al. 2015). This asynchrony remains unexplained, as does the existence of a hypolimnetic cycle absent a solar cue (no measurable light at depth in Trout Bog).

**Fig. 9.** (A) Estimated rates of DOM photomineralization in Sparkling Lake (SP) compared with Trout Bog (TB) based on eq. 1. Solar photon flux obtained for 45°N, 90°W on 13 July from PVLighthouse.  $K_d$  calculated from lake-specific absorption coefficients as per Koehler et al. (2014); absorbance calculated from the absorption coefficient,  $a$ , as  $(1 - 10^{-a(\lambda) \cdot \Delta z / \ln(10)})$ , quantum yield estimated using fit parameters from table 3 in Koehler et al. (2014) (combined data for five lakes). (B) Spectral absorption coefficients for each of the three lakes. Dark band indicates region used to calculate  $S_{275-295}$ .



The second prominent feature of the Sparkling Lake time series was the frequent occurrence of spikes in fluorescence that manifested in the epilimnion as singlets superimposed on the diel cycle (Figs. 4A and 7A). The fluorescence spikes began in early June and dissipated in autumn. Interestingly, they also tended to occur at a frequency of 1 per day and predominantly during late afternoon (Fig. 7B).

Since zooplankton are known to migrate vertically and horizontally with the solar cycle, we conducted a laboratory experiment to empirically determine whether zooplankton swimming into the sensor's light path would enhance, quench, or have no measurable effect on the sensor output. To make this determination on a time scale of minutes rather than days, we used very high concentrations of zooplankton (>1000-fold). The results suggest that crustacean zooplankton indeed fluoresce when excited by UV light (Fig. 8). Given the composition of the crustacean integument (lipoproteins, polyphenols, quinone-tanned proteins), this observation was not wholly unexpected. It is possible, then, that the epilimnetic spikes in fluorescence may be related to diel movements of zooplankton in the lake.

The frequency of fluorescence excursions was much higher in the hypolimnion of Sparkling Lake. After the onset of anoxia, they tended to dominate the hypolimnetic time series (Fig. 4B). They also tended to be negative rather than positive excursions (i.e., quench) with no discernible periodicity. It seems likely that this hypolimnetic "noise" reflects an inner filter effect related to the stratified microbial assemblages clustered near the O–A boundary (Fig. 2; see also Downing et al. 2012). After mixis, the hypolimnetic noise was damped (Fig. 4B).

#### Potential links to photochemistry

Because our prior study ruled out instrumental artifacts, hydrologic forcing, or the effects of inner filtering, pH, or redox conditions (Watras et al. 2015), the two factors most likely driving the diel CDOM cycle are light and microbial activity. Sunlight can affect the quality and quantity of DOM via photobleaching (reduction in molecular mass or aromaticity) or photomineralization (oxidation to dissolved inorganic carbon, DIC) (Sulzberger and Durisch-Kaiser 2009). To constrain the potential effects of photomineralization, we approximated in situ rates using an equation based on Koehler et al. (2014), such that

$$(2) \quad R_p(z) = \sum_{\lambda=300 \text{ nm}}^{600 \text{ nm}} I(\lambda) \cdot e^{-(K_d \cdot z)} \cdot a_*(\lambda) \cdot Q(\lambda) \cdot \Delta\lambda$$

where  $R_p(z)$  is the photomineralization rate at depth  $z$  (mol C·m<sup>-3</sup>·day<sup>-1</sup>),  $I$  is the incident solar photon flux (mol photons·m<sup>-2</sup>·day<sup>-1</sup>),  $K_d$  is the vertical extinction coefficient (m<sup>-1</sup>),  $a_*$  is absorbance (dimensionless), and  $Q$  is the apparent quantum yield (mol C·mol photon<sup>-1</sup>).

The result of simulations under clear-sky, midsummer conditions at this latitude indicate that DOM photomineralization is substantially higher in the near-surface waters of Trout Bog than in Sparkling Lake (Fig. 9A). However, these rates were negligible in the hypolimnia of both lakes and, thus, unable to explain the diel cycles observed there. In the bog, 95% of the total mineralization is confined to the upper 0.5 m of the water column. The high surficial rates result from high light absorbance associated with greater DOM concentrations (Fig. 9B). In Sparkling Lake, our simulations indicate that photomineralization extends much deeper into the water column, with 95% spread out over

the depth interval from 0 to 9.2 m (Fig. 9A). Although estimated rates trail off rapidly with depth in the bog, the surficial rate is sufficiently high to yield a flux somewhat higher than we estimate for Sparkling Lake when integrated over the entire water column ( $2.2 \text{ mmol C}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$  in Trout Bog compared with  $1.7 \text{ mmol C}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$  in Sparkling Lake). Normalized to the mass of DOM in the water column, these areal rates translate to turnovers of  $0.02\%\cdot\text{day}^{-1}$  in Trout Bog and  $0.03\%\cdot\text{day}^{-1}$  in Sparkling Lake.

Because the quantum yield in eq. 2 was derived empirically for complete mineralization (Koehler et al. 2014), the above exercise underestimates the production of photobleached intermediates with different molecular mass, chemical structure, optical properties, and bioavailability than the parent DOM (Sulzberger and Durisch-Kaiser 2009). The spectral slope coefficient ( $S_{275-295}$ ) has been proposed as a useful metric for assessing such changes in natural waters (Helms et al. 2008). Along a transect through Chesapeake Bay, Helms et al. (2008) observed that  $S_{275-295}$  increased as terrigenous DOM was transported via river flow toward optically clear coastal water. Since photo-irradiated water samples showed a similar trend in the laboratory, Helms et al. concluded that photobleaching occurred during downstream transport in the estuary. In other words, the optical and molecular properties of DOM changed progressively with distance from the terrigenous source owing to dilution and increased sunlight penetration. Similar findings have been reported by Fichot and Benner (2012, 2014).

Based on spectral absorbance scans for our study lakes (Fig. 9B), there is evidence that photobleaching may be an important DOM transformation process in Sparkling Lake. In the two bogs,  $S_{275-295}$  averaged  $0.0137 \pm 0.0003$  while in Sparkling lake it averaged  $0.0266 \pm 0.0035$  (Table 1). These values of  $S_{275-295}$  are nearly identical to those reported by Fichot and Benner (2012), who found that  $S_{275-295}$  varied during downstream transport from 0.0139 in humic headwaters to 0.0259 near clear coastal waters. Assuming that terrigenous sources dominate DOM loadings to all three of our study lakes (Wilkinson et al. 2013a; Hanson et al. 2014), and given the larger volume of water subject to solar irradiation in Sparkling Lake due to the low terrigenous flux (Fig. 2A), the inter-lake differences in  $S_{275-295}$  that we observed are consistent with relatively high rates of photobleaching in Sparkling Lake.

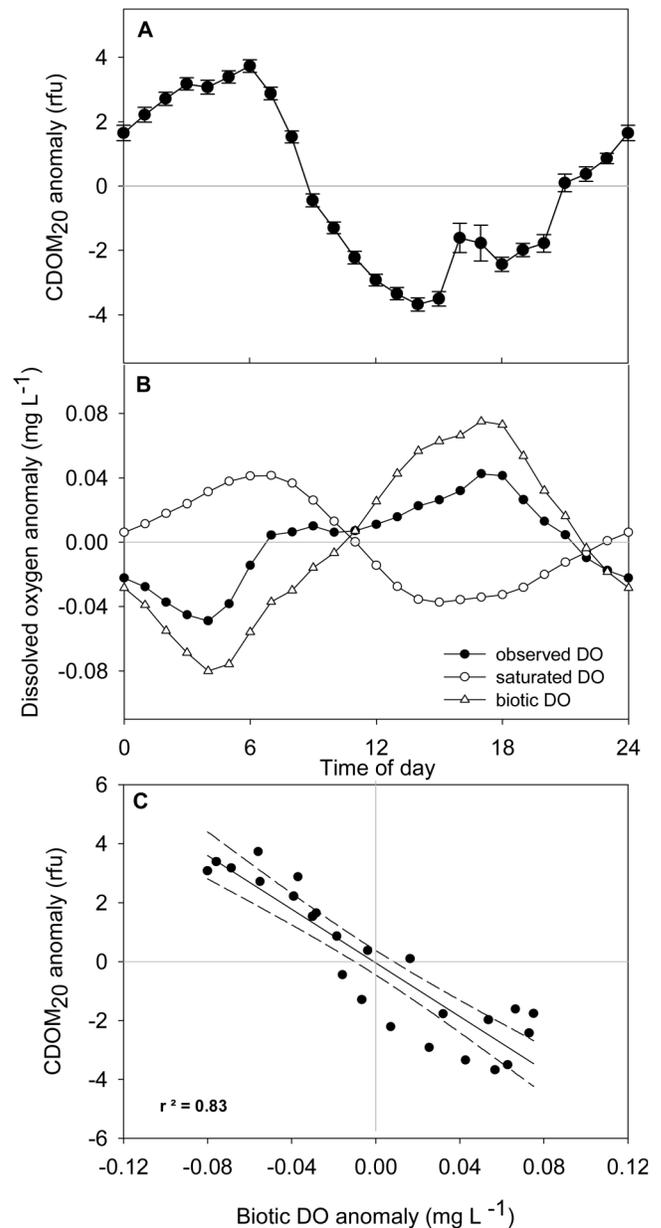
However, neither photomineralization nor photobleaching can account for the diel CDOM cycle observed in hypolimnia where light levels are extremely low. Asynchrony between the epilimnetic and hypolimnetic cycles in Trout Bog suggests the importance of a solar cue (Watras et al. 2015); however, similarities in the timing and magnitude of shallow- and deep-water cycles in Sparkling Lake (Fig. 6) point to the importance of daily oscillations in microbial activity rather than photolysis.

### Potential links to lake metabolism

In our prior study of the bogs, it was argued that properties of the diel CDOM fluorescence cycle can be used to estimate rates of DOM turnover in units of carbon per time (Watras et al. 2015). Given the average magnitude of the diel oscillation ( $\text{rfu}\cdot\text{day}^{-1}$ , peak to trough) and a calibration factor based on the average CDOM fluorescence value and DOM concentration ( $\text{rfu}\cdot\text{mg C}^{-1}\cdot\text{L}^{-1}$ ), we estimate turnover rates of 0.14 and  $0.09 \text{ mg C}^{-1}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$  in the epilimnion and hypolimnion, respectively, of Sparkling Lake (Table 2). Epilimnetic rates in Trout Bog were also higher than hypolimnetic rates ( $0.28$  and  $0.10 \text{ mg C}^{-1}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ , respectively; Table 2), perhaps reflecting a ubiquitous  $Q_{10}$  effect in cold, deep water. Although estimates of the absolute turnover rate were substantially higher in the bog, relative rates were greater in the clear-water lake ( $\sim 5\%\cdot\text{day}^{-1}$ ), perhaps as a result of enhanced bio-availability of the DOM due to photobleaching (Table 2).

In the clear-water lake and the bogs, estimates of DOM turnover based on properties of the diel CDOM<sub>20</sub> cycle were generally consistent with independent estimates based on the daily oscillation

**Fig. 10.** Daily cycles of CDOM<sub>20</sub> fluorescence and dissolved oxygen (DO) in the Sparkling Lake epilimnion. Biotic DO in panel C is the difference between the observed DO and the estimated DO at saturation given temperature and barometric pressure. All data are average values for the time period 1 July – 24 August 2014; bars in panel A are standard error.



of dissolved gases. In the epilimnion of Sparkling Lake, the diel cycles of CDOM<sub>20</sub> fluorescence and DO were anti-correlated (Fig. 10). While CDOM<sub>20</sub> fluorescence rises at night, DO declines because of respiration. During daylight, CDOM<sub>20</sub> fluorescence declines while DO rises because of photosynthesis. On average, the lake is over-saturated with respect to O<sub>2</sub> by about  $0.4 \text{ mg}\cdot\text{L}^{-1}$ . When corrected for daily changes in temperature and barometric pressure, the diel excursion of DO averaged  $\sim 0.16 \text{ mg O}_2\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ , which hypothetically represents the daily biotic flux (Figs. 10B, 10C). Assuming respiratory and photosynthetic quotients of  $\sim 1$  (moles O<sub>2</sub> : moles C) for the metabolic balance  $\text{CH}_2\text{O} + \text{O}_2 \leftrightarrow \text{CO}_2 + \text{H}_2\text{O}$ , we estimate that DOC in the Sparkling Lake epilimnion turns over at a rate of roughly  $0.06 \text{ mg C}^{-1}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$  (Table 2).

A similar estimate of carbon turnover is obtained assuming quasi-equilibrium conditions over a series of days, such that  $\Delta O_2/\Delta t = GPP - R + F_{atm} = 0$ , where GPP is gross primary production, R is respiration, and  $F_{atm}$  is the atmospheric flux of  $O_2$ . Since  $(GPP - R) = \text{net ecosystem production (NEP)}$ , it follows that  $F_{atm} = -\text{NEP}$ . Then, since  $F_{atm} = K_H (O_2 \text{ supersaturation}/Z_{mix})$ , we can estimate NEP using data from the buoy on Sparkling Lake, where  $K_H$  (gas transfer coefficient) =  $1.1 \text{ m}\cdot\text{day}^{-1}$  (Read et al. 2012);  $Z_{mix}$  (the mixed layer depth) =  $3.4 \text{ m}$ ;  $O_2$  observed =  $8.7 \text{ g}\cdot\text{m}^{-3}$ ; and  $O_2$  saturated =  $8.3 \text{ g}\cdot\text{m}^{-3}$ . Using these values, which are averages for time period 1 July to 24 August 2014, we find  $\text{NEP} = 0.13 \text{ mg } O_2\cdot\text{L}^{-1}\cdot\text{day}^{-1}$  or  $0.05 \text{ mg C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ , similar to the two estimates reported above. A comparison of DOC turnover estimates for all three lakes is shown in Table 2 where results for the bogs are from Watras et al. (2015).

### Conclusions

We tentatively conclude that a diel cycle of CDOM<sub>20</sub> fluorescence may be a common property of temperate lakes, and it may provide insight into lake metabolism. The underlying mechanism(s) remain unresolved, but the hypolimnetic cycles point to metabolic rather than photolytic drivers. However, differences in DOM quantity and quality between the clear-water and dark-water lakes imply that terrigenous DOM source strength (e.g., aerial deposition and runoff versus riparian wetland) and consequent differences in solar light attenuation may play important roles in the transformation of relatively refractory DOM to more bioavailable forms, potentially explaining the high relative rates of DOM turnover in clear lakes. The differences between clear and dark lakes may be analogous to the variation in DOM quality and quantity reported along transects from riverine headwaters to coastal ocean waters (e.g., Helms et al. 2008; Fichot and Benner 2012, 2014). However, as noted by Stedman and Cory (2014), our understanding of the interplay of sunlight and microbial activity on DOM processing are incomplete. High-frequency, in situ monitoring of CDOM<sub>20</sub> fluorescence is a promising tool for gaining further insight into transformation processes and potential links to free water metabolism.

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