# Triplet Photochemistry of Effluent and Natural Organic Matter in Whole Water and Isolates from Effluent-Receiving Rivers

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## ABSTRACT

Effluent organic matter (EfOM), contained in treated municipal wastewater, differs in composition from naturally-occurring dissolved organic matter (DOM). The presence of EfOM may thus alter the photochemical production of reactive intermediates in rivers that receive measurable contributions of treated municipal wastewater. Quantum yield coefficients for excited triplet-state OM ($^3$OM*) and apparent quantum yields for singlet oxygen ($^1O_2$) were measured for both whole water samples and OM isolated by solid phase extraction from whole water samples collected upstream and downstream of municipal wastewater treatment plant discharges in three rivers receiving differing effluent contributions: Hockanum R., CT (22% (v/v) effluent flow), E. Fork Little Miami R., OH (11%), and Pomperaug R., CT (6%). While only small differences in production of these reactive intermediates were observed between upstream and downstream whole water samples collected from the same river, yields of $^3$OM* and $^1O_2$ varied by 30-50 % between the rivers. Apparent quantum yields of $^1O_2$ followed similar trends to those of $^3$OM*, consistent with $^3$OM* as a precursor to $^1O_2$ formation. Higher $^3$OM* reactivity was observed for whole water samples than for OM isolates of the same water, suggesting differential recoveries of photo-reactive moieties by solid phase extraction. $^3$OM* and $^1O_2$ yields increased with increasing $E_2/E_3$ ratio ($A_{254nm}$ divided by $A_{365nm}$) and decreased with increasing electron donating capacities of the samples, thus exhibiting trends also observed for reference humic and fulvic acid isolates. Mixing experiments with EfOM and DOM isolates showed evidence of quenching of triplet DOM by EfOM when measured yields were compared to theoretical yields. Together, the results suggest that effluent contributions of up to 25% (v/v) to river systems have a negligible influence on photochemical production of $^3$OM* and $^1O_2$ apparently because of quenching of triplet DOM by EfOM. Furthermore, the results highlight the importance of whole water studies for quantifying *in situ* photoreactivity, particularly for $^3$OM*.

## INTRODUCTION

Urbanization increases contributions of treated municipal wastewater effluent to streams and rivers. Almost one quarter of permitted wastewater discharges now occur into rivers and streams with dilution factors of ten or less.[1] Municipal wastewater discharges are a source of synthetic organic micropollutants (*e.g.*, pharmaceuticals and many personal care products) that can adversely impact downstream ecosystem health.[2-5] Downstream concentrations of micropollutants may be attenuated by biological degradation or photochemical reactions.[6-8] In the latter case, aquatic dissolved organic matter (DOM) plays a dual role by slowing direct photolysis reactions[9] while simultaneously generating photochemically-produced reactive intermediates that can enhance micropollutant degradation through secondary reactions.[9-12] These photochemically-produced reactive intermediates include triplet-state excited organic matter ($^3$OM*), singlet oxygen ($^1O_2$), and hydroxyl radicals (OH•) that are both produced[13-18] and scavenged by DOM, yielding low steady state concentrations in natural waters.[18-22] Photoproduction of reactive intermediates may be altered by large fractional contributions of treated wastewater, which introduces effluent organic matter (EfOM) that differs in chemical composition from upstream and in-stream sources.[23,24] To date, the photoreactivity of treated wastewater EfOM has not been studied extensively[25,26] and how it may impact the downstream fate of wastewater micropollutants is poorly understood.[10,27]

The composition of EfOM differs from that of naturally-occurring DOM in fluvial systems in ways that are expected to affect aquatic photochemistry. The photoreactivity of organic matter is often assessed in relation to its optical properties, including specific ultraviolet absorbance (*e.g.*, at 254 nm, $SUVA_{254}$) and slope characteristics of the absorbance spectrum.[25,28-31] EfOM, in comparison to DOM, exhibits lower $SUVA_{254}$ values[24,32-34] and therefore absorbs, or attenuates, less light on a per carbon basis in the water column than DOM. The ratio of DOM absorbance at 254 nm to 365 nm ($E_2/E_3$ ratio) has been related to the quantum yields of photochemically-produced reactive intermediates.[29,30] For example, the apparent quantum yield of $^1O_2$ is positively correlated with $E_2/E_3$ values of isolated DOM, International Humic Substance Society (IHSS) reference materials, and whole water samples.[29,30] This relationship has been extended to isolated EfOM; however, EfOM apparent quantum yields of $^1O_2$ are somewhat higher than those for IHSS reference materials[25] consistent with higher $E_2/E_3$ values typically observed for EfOM relative to DOM.[24] Higher $^1O_2$ yields from EfOM suggest that quantum yields of $^3OM^*$ are also greater for EfOM than for DOM because $^3OM^*$ is thought to be the precursor for $^1O_2$ formation.[35,36] $^3OM^*$ yields of EfOM have not been reported previously but can be inferred to be higher than those of DOM from enhanced triplet state reactivity and degradation of sulfamethoxazole in treated municipal wastewater relative to lake water.[10,37] Together, these observations suggest that wastewater treatment plant discharges may considerably influence the formation of photochemically-produced reactive intermediates in effluent-receiving rivers, with the actual influence depending upon the relative mass contributions of EfOM and DOM to the river, as well as the relative specific absorbances of the OM from these two sources.

An important consideration in studies of DOM and EfOM photochemistry is the extent to which the sampling and isolation of the OM may alter its composition and influence subsequent photochemical properties. Although isolation techniques often have highly variable overall recoveries, the isolated OM still contains a significant fraction of chromophoric, photoreactive components.[38] In contrast, isolation techniques may not capture all of the photoreactive components of EfOM. Isolation of EfOM by solid phase extraction typically results in overall recoveries that are on the low end of the range reported for DOM. For example, XAD-8 resin extraction only captures 20 to 40% of the total EfOM on a carbon basis.[24,32,34,39,40] Importantly, lower molecular weight organic matter (< 1 kDa, membrane separation) that may be less effectively captured by resin isolation shows greater apparent $^1O_2$ quantum yields than larger molecular weight fractions[25] (> 10 kDa, membrane separation) that could be more effectively captured by resin isolation. More recently, the availability of wide-polarity spectrum solid phase extraction materials such as styrene divinylbenzene (trademarked as PPL) have been shown to capture a much larger fraction of the organic matter pool (> 50%),[41,42] but application to EfOM has not been investigated extensively. Although many past studies of DOM photoreactivity have been conducted using isolates, it is not clear whether the photoreactivity of solutions prepared with isolates is truly representative of whole water samples,[43,44] particularly those with effluent contributions.

The purpose of this study was to investigate the influence of EfOM discharges on $^3OM^*$ and $^1O_2$ photoproduction in stream and river systems receiving moderate amounts of treated municipal wastewater effluent, as typical for the Northeast and Midwest U.S. We used whole water samples and solutions prepared with OM isolated from water samples by solid phase extraction. The water samples were collected up- and downstream of the treated wastewater outfalls. In addition, we used solutions prepared with EfOM isolated from the wastewater treatment plant. This approach was designed to examine any bias that might result from the use of DOM isolates, relative to unaltered DOM in whole water samples. To our knowledge, $^3OM^*$ has not been studied previously in wastewater effluent or EfOM isolates, despite it being an important photooxidant for micropollutants from many substance classes.[9,10,13,37,45-47] Furthermore, we sought to examine whether apparent quantum yields of $^1O_2$ and triplet OM vary in similar ways with OM source in order to verify the assumption that triplet OM is the precursor to $^1O_2$[35,36] and to examine whether probes for the two species provide consistent information on the production of triplet state photochemistry. Finally, we evaluated the relationships of the respective quantum yields to OM properties, including $E_2/E_3$ ratios and OM isolate electron-donating and accepting capacities, to assess whether previously reported relationships for DOM also applied to EfOM and natural waters receiving treated wastewater effluent discharges.

## MATERIALS AND METHODS

### Sample Collection and Preparation

Three wastewater treatment plant (WWTP) sites with different amounts of municipal effluent contributions were examined: (i) Hockanum River (Vernon, CT), 22% effluent by volume at the downstream sampling site; (ii) East Fork Little (EFL) Miami River (Batavia, OH), 11 volume % effluent, and (iii) Pomperaug River (Southbury, CT), 6 volume % effluent. The volumetric fraction of effluent discharged to each river was determined from boron dilution ratios.[48] Each site receives wastewater primarily from residential sources with minor inputs from commercial businesses. All of the plants employ conventional activated sludge treatments with some variations in operation. The Hockanum R. plant (4 million gallons per day, MGD) has secondary treatment with the addition of powdered activated carbon to the activated sludge tanks (PACT® process) and does not have advanced nitrogen removal. The EFL Miami R. plant (3.2 MGD) is a conventional secondary activated sludge treatment plant and also has no advanced nitrogen removal. The Pomperaug R. plant (0.4 MGD) has no primary treatment and oxygen delivery to the activated sludge tanks is pulsed to allow denitrification to occur during periods of anaerobic operation. Effluent from the Pomperaug R. plant is discharged to two oxidation ponds in series before being discharged into the river. Prior characterization of EfOM isolated from the two CT WWTP in 2010 and 2011 showed only small differences in their bulk characteristics, despite differing plant operating conditions.[24]

To assess the impact of effluent contributions on the photochemical generation of reactive species in the rivers, whole water samples were collected at each site in summer 2013 from two river locations, the first upstream of the WWTP outfall and the second downstream where the effluent plume was well-mixed across the river, based on boron and conductivity measurements. Aliquots of these samples were filtered through 0.45 µm PTFE membrane filters (Whatman) and used for whole water experiments. An additional 250 mL of filtered whole water was used for small-scale organic matter isolation. Separate large-scale organic matter isolation of DOM and EfOM was undertaken for paired upstream and effluent samples (each 20 L), respectively, collected in summer 2012 from the Hockanum R., EFL Miami R. and in summer 2013, from the Pomperaug R. As detailed in the Supporting Information, isolates were obtained by solid phase extraction using PPL stationary phases (styrene divinylbenzene), eluted with methanol and evaporated to dryness.[41] Small-scale isolates were immediately dissolved in buffer solution, as described below, while large-scale isolates were dissolved in high purity water and freeze-dried. We note that similarities in yields of triplet organic matter and singlet oxygen species for both the small and large-scale 2013 OM isolates from the Pomperaug R. (Table S1) strongly suggests that the scale at which DOM was isolated did not affect its photoreactivity.

**Photochemistry Experiments**

Photochemistry experiments were conducted in two different reactors located at the University of Connecticut and the University of Mary Washington (see SI for details). Both reactors were configured so that the majority of photochemically-active radiation reaching the samples was centered around 365 nm. The intensity of the radiation reaching the samples was determined by *p*-nitroanisole/pyridine actinometry.[49] Light intensities were typically about $2.6 \times 10^{-5}$ Es $L^{-1}$ $s^{-1}$ at Connecticut and $2.4 \times 10^{-5}$ Es $L^{-1}$ $s^{-1}$ at Mary Washington, and sample quantum yields measurements of split samples with both reactors were in good agreement (Figure S2).

Quantum yields were determined from degradation of probe compounds spiked to the samples from aqueous stock solutions. Probe compounds were 2,4,6-trimethylphenol (TMP, 5 μM initial concentration) for $^3OM^*$[50] and furfuryl alcohol (FFA, 25 μM initial concentration) for $^1O_2$.[51] Individual probes were spiked directly into filtered whole water samples under natural pH conditions (6.8 – 7.5, Table 1). Samples of OM isolates were prepared by diluting stock solutions (see SI) with 10 mM phosphate buffer at the desired pH (pH 8 for $^3DOM^*$ experiments and pH 6.9 for $^1O_2$ experiments) for consistency with previous studies.[14,29,36] The final dissolved organic carbon (DOC) concentrations in experimental solutions are given in Table 1 and were quantified by high temperature oxidation (see SI). For comparison, nine solutions were prepared from International Humic Substance Society reference materials (see SI for complete list; all at 5 $mg_C$ $L^{-1}$) in 10 mM phosphate buffer for both $^3DOM^*$ and $^1O_2$ experiments. Changes in probe concentrations over time were measured by HPLC using isocratic elution with a C-18 column and UV absorbance detection (see Table S2 for operating conditions). The loss of the probe was used to determine quantum yield coefficients for $^3OM^*$ ($f_{TMP}$) and apparent quantum yields for $^1O_2$ ($\Phi_{1O2}$) following standard approaches (see SI for calculation details). Quenching experiments with isopropanol were conducted with whole water samples to confirm that 2,4,6-trimethylphenol loss was dominated by reaction with $^3OM^*$. Less than approximately 10% quenching was observed indicating that there was either a small contribution to TMP reactivity by OH•, or slight quenching of $^3$OM* by isopropanol[52] (Figure S3).

### Optical Analyses

Absorbance spectra were collected from 200 to 550 nm in 1-cm quartz cuvettes on a double beam UV/Vis spectrophotometer (Agilent Cary 50) using a 1 nm slit. Blank corrections were applied by subtracting high purity $H_2O$ absorbance spectra from whole water samples, and buffer solution spectra from isolate solutions. $E_2/E_3$ ratios were obtained by dividing the absorbance at 254 nm by the absorbance at 365 nm[53] and specific absorbance ($SUVA_{254}$) values ($L\ mg_C^{-1}\ m^{-1}$) were calculated by dividing the absorbance at 254 nm by the DOC concentration.[54] OM fluorescence spectra were measured (Cary, Eclipse, Agilent) using the procedures of McKnight et al.[55] to construct excitation-emission matrices (EEMs), as detailed in the SI. Fluorescence indices were calculated for excitation at 370 nm from the ratio of the emission intensity at 450 nm to that at 500 nm.[55]

### DOM Electron Donating and Accepting Capacities

The electron donating and accepting capacities (EDC and EAC) of large-scale OM isolates collected in 2012 were determined following the method of Aeschbacher et al.,[56,57] in an anoxic glove box by mediated electrochemical oxidation and reduction, respectively. Measurements were made under the same conditions as reported previously for reference organic matter samples.[56] EAC and EDC values of seven IHSS reference materials were included from previous work[56] for comparison with the OM isolates used in this study. Details on the electrochemical measurements are reported in the SI.

## RESULTS AND DISCUSSION

### Influence of EfOM on Whole Water Optical Properties

The EfOM inputs at each site can be gauged by comparing DOC concentrations of the water above and below the WWTP location (Table 1). Whole water samples from below the WWTP outfalls had DOC levels that were not significantly different from samples taken upstream of the treatment plants (± 0.2 mg/L). This observation suggests that EfOM concentrations in the treated effluent at the time of sampling (measurements not obtained) were comparable to the DOM concentrations reported for upstream river samples, a trend we have observed in previous sampling rounds. Thus, at the time that samples were collected, effluent contributions by volume, as calculated from boron dilution ratios, are also indicative of the fractional EfOM mass contribution to the overall organic matter concentrations downstream of the WWTPs.

We examined the optical properties of whole river water samples collected upstream and downstream of the WWTPs to assess potential changes due to EfOM contributions. Based on the known properties of isolated EfOM relative to DOM, differences in the optical properties of the water above and below the WWTP could be anticipated. These differences include decreases in $SUVA_{254}$ and increases in $E_2/E_3$ and fluorescence index values.[24,32-34] In the Hockanum River, $SUVA_{254}$ decreased and $E_2/E_3$ increased and hence, changed as expected for EfOM contributions to the optical properties (Table 1). Furthermore, excitation-emission matrices (EEMs) of water collected downstream of the WWTP outfall on the Hockanum R. had a humic 'C' peak that aligned with the 'C' peak measured in the effluent, but not with the 'C' peak of the upstream water where emission was observed at longer wavelengths (Figure S5), also indicating effluent contributions to the optical properties of downstream water. Natural variations in $SUVA_{254}$ and $E_2/E_3$ values for the Hockanum R. across sampling dates in July 2013 and May (12 % v/v effluent) and September 2014 (24 % v/v effluent) were greater than analytical uncertainty (Table S3); in contrast for each sampling date, similar trends (lower $SUVA_{254}$, higher $E_2/E_3$) were observed between whole water from downstream and upstream locations (Table S3). For the other two rivers, no significant differences were observed between the optical properties of the whole water samples collected upstream and downstream of the WWTPs (Table 1).

In contrast to the whole waters, OM isolated from upstream and downstream of the WWTP outfalls showed clear differences in optical properties. Isolates downstream of the WWTP discharges in the Hockanum R. and EFL Miami R. showed lower $SUVA_{254}$ and higher fluorescence index values, compared to the respective OM isolates from upstream of the WWTP (Table 1). These observations suggest that different subcomponents of DOC were isolated by solid phase extraction at the upstream and downstream locations: the optical properties of the downstream isolates suggest that these had larger contributions of EfOM – which has comparatively low $SUVA_{254}$ and high fluorescence index values – than its mass contribution to the OM in the whole water sample.

## Quantum Yield Measurements

For the OM isolates, we first determined the yields of $^3OM^*$ and $^1O_2$ formation as a function of DOC concentration using a dilution series to ensure that the measured results were not biased by potential self-quenching of reactive intermediate production by the organic matter.[21,58-61] For EfOM isolates, a strong inhibition of TMP oxidation was observed with increasing DOC: as DOC increased from 3 to 25 $mg_C\ L^{-1}$, $f_{TMP}$ decreased by 50-60% (Figure 1; B1,B2). In stark contrast, DOM isolated from upstream of the WWTP outfalls showed little variations in $f_{TMP}$ with DOC (Figure 1; A1, A2). To our knowledge, this is the first observation of a self-quenching effect of EfOM on $^3EfOM^*$ induced oxidation, and this inhibition occurs at much lower DOC concentrations than reported for reference materials.[21] The cause of the strong inhibiting effect of the EfOM, as compared to the DOM, remains unidentified. Two possible scenarios could cause a decrease in $f_{TMP}$ with increasing EfOM. There could be direct self-quenching of $^3EfOM^*$ by ground state EfOM. This explanation requires that $^1O_2$ is produced by photoexcitation of different OM species than are responsible for TMP oxidation given that we observed a much smaller relative decrease in $\Phi_{1O2}$ than in $f_{TMP}$ over the same DOC range (Figure S6). The $\Phi_{1O2}$ result is consistent with prior reports of negligible $^1O_2$ quenching by DOM isolates.[20,51,62] However, the discrepant trends between $\Phi_{1O2}$ and $f_{TMP}$ are difficult to reconcile with recent evidence that strongly ties TMP oxidation to triplet states whose lifetimes are controlled by $O_2$[63] and that are responsible for $^1O_2$ production.[36] An alternative explanation to self-quenching is inhibition of TMP oxidation via interference by EfOM in secondary reactions of the TMP phenoxyl radical that lead to overall TMP loss. Possible interferences include reduction of the TMP radical by EfOM,[58,59,64] or EfOM scavenging of superoxide, a potentially important intermediate in the TMP oxidation mechanism.[61,65] We chose 5 $mg_C L^{-1}$ as our working concentration for subsequent experiments with isolates because most of the whole water samples had similar DOC concentrations (Table 1), despite the fact that slight quenching effects were observed at this concentration level.

**Quantum Yields in Whole Water Samples.** With one exception, effluent discharges caused little changes in $f_{TMP}$ and $\Phi_{1O2}$ in the river waters as indicated by similar downstream and upstream values of these parameters (Figure 2; A1, A2). The only exception was the Pomperaug R., which showed a decrease of about 50% in $f_{TMP}$, from samples collected upstream to samples collected downstream of the WWTP (Figure 2; A1). Given the small contribution of EfOM to the overall DOC in this river (Table 1), it seems unlikely that the change in $f_{TMP}$ was due to inhibition of $^3OM^*$ by EfOM (6% mass contribution). Of the three river systems, the EFL Miami R. had the highest $f_{TMP}$ and $\Phi_{1O2}$ values, 30 to 50% larger than the corresponding values of samples from the two CT sites (Figure 2; A1, A2). The $f_{TMP}$ and $\Phi_{1O2}$ values of the samples collected both upstream and downstream of the WWTP are within ranges reported previously for natural water samples.[29,30,66-68] Overall, our results suggest that modest EfOM contributions to river systems are unlikely to impact the downstream production of $^3DOM^*$ and $^1O_2$.

**Quantum Yields for Organic Matter Isolates.** In this work, we also compared the formation of $^3OM^*$ and $^1O_2$ between whole water samples and solutions prepared with organic matter isolates obtained from aliquots of the same water. Quantum yields are commonly measured using isolated DOM due to its stability and the overall convenience of this approach.[44] Solutions prepared from paired OM isolates collected upstream and downstream of each WWTP showed little differences in $f_{TMP}$ and $\Phi_{1O2}$ values (Figure 2), as was the case for whole water samples. Notable differences were observed in $f_{TMP}$ values between whole waters and their respective OM isolate solutions, with smaller $f_{TMP}$ values of isolate solutions than of corresponding whole waters (Figure 2; A1 vs B1). DOC concentrations were closely matched between the samples (Table 1) to minimize possible DOC-dependent inhibitory effects. In contrast, we did not observe lower yields for $^1O_2$ formation in isolate samples than in the corresponding whole waters. Rather, $\Phi_{1O2}$ values for OM isolates were slightly higher (Hockanum R.) or comparable (EFL Miami R., Pomperaug R.) to those of the corresponding whole water samples (Figure 2; A2 vs B2). The finding, that $f_{TMP}$ was lower in isolates than whole waters while $\Phi_{1O2}$ was similar, contrasts with the expected result of similar trends in these parameters. Such an expectation is based on recent results suggesting that the same pool of $^3$DOM* is believed to contribute to both $^1O_2$ formation and TMP oxidation.[36,69] If this is true, then the difference in $f_{TMP}$ and $\Phi_{1O2}$ trends between the isolates and whole waters cannot be explained by incomplete recovery of triplet precursors in the isolation procedure. An alternative explanation is that species capable of oxidizing TMP were present in the whole water but not the isolates. We can rule out $^1O_2$ or OH• as possible TMP oxidizers, given that $\Phi_{1O2}$ values for OM isolates were larger than, or similar to, those of the whole waters (Figure 2; A2, B2) and that control experiments with whole water samples showed that $f_{TMP}$ values decreased only slightly when adding isopropanol as an OH• quencher (Figure S3). A final explanation is that there are higher concentrations of OM species capable of inhibiting TMP oxidation in the isolate solutions than in the whole waters.[58,59] While the cause for lower $f_{TMP}$ values in OM isolates than whole water OM samples remains unidentified, the observed differences highlight that one needs to be cautious when using results from experiments conducted with solid phase extraction-isolated OM to predict OM photoreactivity in unaltered whole waters. Furthermore, it is possible that the transformation rates of compounds with complex overall oxidation mechanisms, including TMP, are altered by components in the whole water samples that are not present in solutions prepared from OM isolates (*e.g.*, unrecovered fractions of OM or other possible oxidants).

**Simulated Mixing Scenarios.** We examined apparent quantum yields for solutions prepared with mixtures of isolated EfOM and DOM from upstream of the respective WWTP to assess how different sources of organic matter may affect $^3$OM* and $^1O_2$ formation for volumetric mixing ratios of effluent and river waters other than those occurring at our field sites (Figure 3). Comparison of isolated EfOM and upstream DOM from the same river showed greater $f_{TMP}$ and $\Phi_{1O2}$ for EfOM than DOM in the Hockanum R., consistent with differences previously reported in the literature.[10,25] Clear increases in $f_{TMP}$

and $\Phi_{1O2}$ values with increasing EfOM addition were observed for DOM from the Hockanum R. when the total DOC was kept constant at 5 $mg_C$ $L^{-1}$ (Figure 3; A1, A2). Here, mass contributions of isolated EfOM of 50% or larger resulted in higher $f_{TMP}$ values than for the isolated DOM (Figure 3; A1). Importantly, samples in which EfOM constituted 25% of the DOC (Figure 3; A1) mimic the OM composition of the Hockanum R. downstream of the WWTP when whole waters were sampled. For this case, similar $f_{TMP}$ values were obtained for the EfOM/DOM isolate mixture and for the pure DOM isolate (Figure 3; A1), consistent with the similar values observed for $f_{TMP}$ between OM isolates and whole water samples obtained upstream and downstream of the Hockanum R. WWTP (Figure 2; A1, B1), despite the much higher photoreactivity of EfOM compared to DOM from this location. The $\Phi_{1O2}$ values were slightly higher in the simulated isolate mixture with 25% EfOM as compared to the isolated DOM (Figure 3; A2), which is further consistent with the differences seen with the upstream and downstream OM isolates (Figure 2; A2 and B2). For the EFL Miami R., $f_{TMP}$ and $\Phi_{1O2}$ values for the EfOM isolate were much closer to those of the upstream DOM isolate. As a consequence, no clear changes in either $f_{TMP}$ or $\Phi_{1O2}$ were observed with increasing contributions of EfOM to mixtures of OM isolates (Figure 3; B1 and B2). Pomperaug R. EfOM and DOM isolate $f_{TMP}$ values (130.8 ± 30.8 $M^{-1}$; 48.6 ± 3.8 $M^{-1}$, respectively) and $\Phi_{1O2}$ values (5.0 ± 0.8 %; 3 ± 0.3 %, respectively) showed higher photoreactivity of EfOM; however, mixing experiments were not conducted because of the low effluent inputs to the Pomperaug R.

As discussed in detail below, the photochemistry observed in EfOM and DOM isolate mixtures does not follow patterns expected for conservative mixing. This suggests that EfOM quenching of DOM photoreactivity may explain the observed lack of an effect of WWTP effluent on $f_{TMP}$ and $\Phi_{1O2}$ in our whole water experiments. Ultimately, the extent to which OM from a given source contributes to the observed quantum yields in an OM mixture depends upon both the photoreactivity of the individual OM components and the fraction of light absorbed by the individual components. Unfortunately, the available field data (*i.e.*, measured volumetric and DOC mass mixing ratios of effluent with river water) do not include absorption spectra of the WWTP effluent, so it is not possible to calculate the expected apparent quantum yields in the whole water samples downstream of the WWTP outfall. In contrast, the photoreactivity of EfOM and DOM isolate mixtures can be estimated based on the optical characteristics of the pure EfOM and DOM isolates (Figure 3). The apparent quantum yield, $\Phi_{i,app}$, of a mixture can be calculated from the ratio of the overall production of species *i* to the overall rate of light absorption of the sample by assuming no interactions of the two photoreactive components (*i.e.*, conservative mixing):

$$\Phi_{i,app} = \frac{\left(1-10^{-\propto_1 DOC_1 z}\right)\Phi_{i,1}+\left(1-10^{-\propto_2 DOC_2 z}\right)\Phi_{i,2}}{\left(1-10^{-(\propto_1 DOC_1 + \propto_2 DOC_2)z}\right)} \qquad (1)$$

where $\Phi_{i,1}$ and $\Phi_{i,2}$ are the apparent quantum yield of *i* for the individual components 1 and 2, $\alpha_1$ and $\alpha_2$ are the specific absorption coefficients of the two waters (L $mg_C^{-1}$ $cm^{-1}$), $DOC_1$ and $DOC_2$ are the OM concentrations of components 1 and 2 ($mg_C$ $L^{-1}$), and *z* is the optical path length (cm). Calculated $f_{TMP}$ and $\Phi_{1O2}$ values for the Hockanum R. are shown in Figure 3 using specific absorption coefficients obtained at 365 nm of 0.0029 and 0.0048 L $mg_C^{-1}$ $cm^{-1}$, respectively, for EfOM and DOM isolates. Calculated $f_{TMP}$ and $\Phi_{1O2}$ values were always larger than measured values for the isolate mixtures (Figure 3; A1, A2), indicating that conservative mixing did not hold. Given that absorbance values of the EfOM:DOM isolate mixtures were consistent with predicted values, the lower than predicted $f_{TMP}$ and $\Phi_{1O2}$ values suggest that EfOM quenched triplets of DOM in the mixtures. For the EFL Miami R., EfOM and DOM isolates had similar $f_{TMP}$ and $\Phi_{1O2}$ values (Figure 3; B1, B2) (and similar specific absorbances at 365 nm; 0.0072 L $mg_C^{-1}$ $L^{-1}$ for EfOM and 0.0078 L $mg_C^{-1}$ $L^{-1}$ for DOM) such that the different mixtures were expected to show comparable photoreactivities. Nonetheless, the mixture of 25/75 EfOM and DOM isolates for this river – which most closely matches the water downstream of the WWTP outfall – had a lower $f_{TMP}$ value than the pure DOM isolate (Fig. 3, B1), indicating the possibility that there could be EfOM quenching of DOM triplets in the downstream whole water. Together, the comparison of measured and calculated apparent quantum yields for OM mixtures suggests that the lack of differences in upstream and downstream $^3$DOM* and $^1O_2$ production in whole water samples may be attributed to quenching of triplet state DOM by EfOM, particularly in the case of the Hockanum R. that received the largest OM mass contributions from treated municipal wastewater.

## Correlation of Quantum Yields with $E_2/E_3$ Ratios

To expand on recent reports[25,29,30,67,69] and to provide additional comparisons between EfOM and DOM photochemical reactivity, we examined trends between optical properties ($E_2/E_3$) and photoreactivity for whole waters and organic matter isolates. A positive correlation between $\Phi_{1O2}$ and $E_2/E_3$ for organic matter samples was reported previously[25,29,30,67,69] and attributed to the efficiency of $^3$DOM* formation from OM precursor moieties. It is hypothesized that intermolecular complexes between electron-donating and accepting moieties in OM result in long-wave absorption (low $E_2/E_3$) that is inefficient at producing triplet state photochemistry.[67] Although correlations between $f_{TMP}$ and $E_2/E_3$ ratios have not been examined in the literature, a positive correlation should exist because of the fact that $^1O_2$ is produced by $^3$DOM*. Indeed, both $f_{TMP}$ and $\Phi_{1O2}$ correlated positively with $E_2/E_3$ for our complete sample set (Figure 4). Previously published values[25] of $\Phi_{1O2}$ for effluent whole water samples and $E_2/E_3$ ratios also were within the bounds of our correlation (Figure 4, '×'). Furthermore, all the samples examined here (whole water, OM isolates, and EfOM/DOM mixtures) showed trends of $f_{TMP}$ and $\Phi_{1O2}$ with $E_2/E_3$ that were similar to the ones observed for reference fulvic and humic acids (Figure 4). Notably, despite the differences in absolute values of $f_{TMP}$ for whole water samples and their corresponding DOM isolates, $f_{TMP}$ for both sample types showed similar relationships to $E_2/E_3$. The similar trends in $f_{TMP}$ and $\Phi_{1O2}$ with $E_2/E_3$ for isolated OM and whole water samples – both parameters increasing by a factor of approximately 10 over the same $E_2/E_3$ range (Figure 4) – reveal a subset of $^3$OM* and $^1O_2$ precursors that were simultaneously isolated by solid phase extraction and likely have a high degree of overlap in their reactivities.[36,69] Our results corroborate previous reports[25,29,30] that proposed that $E_2/E_3$ may be a simple parameter to estimate apparent quantum yields of $^3$DOM* and $^1O_2$ (and hence, production rates and steady-state reactive species concentrations) in cases that photochemical determinations are not feasible or impossible. We note a caveat in cases when DOM samples undergo photooxidation for which destruction of sensitizing chromophores increases $E_2/E_3$ but decreases $f_{TMP}$[67];

however, this should be more relevant for open water bodies because OM in rivers is less subject to photooxidation due to shorter residence times.

## OM Redox Properties and Photochemistry

To extend our knowledge of how photochemical reactivity is linked to chemical composition of EfOM and DOM, we also explored relationships between quantum yields and electron-donating and accepting capacities (EDC and EAC) of large-scale EfOM and DOM isolates. DOM substructures suspected to be involved in photoreactivity (phenols and quinones)[69,70] are also thought to be active in electron transfer.[56,57] Recently, quantum yields of $^1O_2$ have been shown to correlate negatively to EDC for several OM samples undergoing photooxidation.[67] Such a relationship was also observed here between EDC and both $f_{TMP}$ and $\Phi_{1O2}$ for isolated EfOM and DOM, and a similar trend was observed with reference fulvic and humic acids (Figure 5). These trends are consistent with the current model for the structural basis of DOM photochemistry, *i.e.*, that electron donating moieties participate in charge transfer complexes[29,69,71] and that the formation of $^3$DOM* is impaired in DOM that has a high abundance of these complexes. We also note that neither $f_{TMP}$ nor $\Phi_{1O2}$ showed a correlation with EAC (results not shown), which is consistent with a report by Sharpless et al.,[67] who argued that a weak relationship of these properties to EAC indicates only a minor role for quinones in $^3$DOM* photochemistry of aquatic DOM.[67] Collectively, these findings suggest that photoreactivity in OM isolated by solid phase extraction correlates negatively with the concentration of redox active substructures and further suggest that aromatic ketones, rather than quinones, are primary triplet sensitizers in these OM isolates.

## Environmental Implications

When placed in the context of treated municipal wastewater discharge scenarios, our findings suggest that effluent may have a smaller influence on downstream photochemistry than previously anticipated. Mostafa et al.[25] and Dong et al.[26] showed EfOM to have greater quantum yields of $^1O_2$ and

OH• than for reference fulvic and humic materials. If our $f_{TMP}$- and $\Phi_{1O2}$-$E_2/E_3$ ratio relationships are indicative, there is likely overlap between the photoreactivity of EfOM and that of DOM from natural waters. Values of $E_2/E_3$ for EfOM are not widely reported; literature values, including our study, of $E_2/E_3$ ratios for EfOM range from 4 to 8 (n = 11, 7 WWTP)[24,25] with most values between 4.8 and 5.7. EfOM characterized by the lower range of $E_2/E_3$ ratios (< 5) may have similar photoreactivity as receiving water DOM that we, and others,[29,30] have found to have greater $E_2/E_3$ ratios than some commonly studied reference fulvic and humic materials. According to Eq. 1, the maximum influence of EfOM discharges on downstream apparent quantum yields depends on the relative values of $f_{TMP}$ and $\Phi_{1O2}$ of the EfOM and DOM, their fractional contribution to the total DOC contribution in the mixture and the relative magnitude of the EfOM and DOM absorption coeffieicnts, which are fixed for a particular combination of OM sources. However, as shown above, conservative mixing estimates of downstream apparent quantum yields are complicated by what appears to be quenching of triplet states by EfOM, which reduces the effective EfOM contribution to overall photochemical reactivity. Typical effluent DOC concentrations range from 5 to 20 $mg_C$ $L^{-1}$.[72] For low DOC effluents, effects on downstream photoreactivity will likely only occur for conditions of both high mass contributions (volumetric discharge) and high EfOM $E_2/E_3$ ratios (indicative of high triplet photoreactivity). However, even under these conditions, it is possible that EfOM self-quenching will greatly suppress EfOM photoreactivity. Relationships, such as shown in Figure 4, do suggest promise for using mixture $E_2/E_3$ ratios to estimate photoreactivity provided that they are constructed under a representative DOC concentration (Figure S7). Studies including a broader array of ecosystems should be undertaken to validate the predictive ability of this approach and to assess whether the trends in apparent quantum yields reported here are, or are not, ecosystem dependent.[67]

Furthermore, our results suggest that the use of TMP as a probe can lead to $^3OM^*$ quantum yields that are higher in whole water than for samples prepared with OM isolates from the same water samples. Additional study is warranted to evaluate whether isolated DOM (by XAD 8 or other solid phase extraction methods) should be used in $^{3}$OM* reactivity studies and the extent to which the choice of probe compound may influence the results.[14,44,64]

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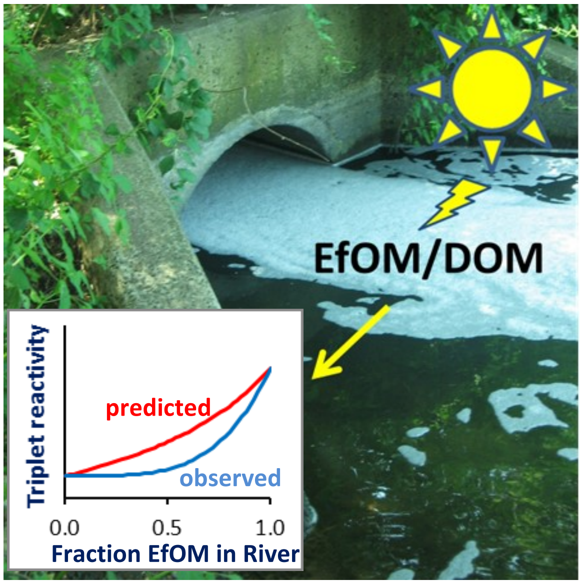
## Supporting Information Available

Supporting Information contains additional details about organic matter isolation, photoreactors, International Humic Substance Society reference organic matter materials, sample analysis and yield calculations. This information is available free of charge via the Internet at http://pubs.acs.org/.

## TOC Art



*Photograph was taken by Allison A. MacKay.*

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**Table 1. Water quality and optical properties of whole waters and solutions prepared with OM isolates (small-scale) collected in July 2013. Parameters for large-scale OM isolates are italicized.**

| Sample | pH | [DOC][a] (mg $L^{-1}$) | $SUVA_{254}$[b] (L $mg_C^{-1}$ $m^{-1}$) | $E_2/E_3$[c] | Fluores-cence Index[d] |
|---|---|---|---|---|---|
| Hockanum River (22% (v/v) effluent flow) | | | | | |
| Whole water upstream | 6.8 | 4.5 | 5.5 | 4.1 | 1.5 |
| Whole water downstream | 7.5 | 4.5 | 5.1 | 4.4 | 1.4 |
| OM isolate upstream | 8.0/6.9[e] | 4.0 | 4.4 | 4.4 | 1.7 |
| OM isolate downstream | 8.0/6.9 | 5.0 | 2.7 | 4.9 | 2.3 |
| *2012 DOM isolate upstream* | *8.0/6.9* | *5.0* | *2.6* | *5.3* | *1.2* |
| *2012 EfOM isolate* | *8.0/6.9* | *5.0* | *2.0* | *7.2* | *1.9* |
| EFL Miami River (11% (v/v) effluent flow) | | | | | |
| Whole water upstream | 7.2 | 6.2 | 2.9 | 5.2 | 1.3 |
| Whole water downstream | 7.1 | 6.5 | 2.8 | 5.2 | 1.5 |
| OM isolate upstream | 8.0/6.9 | 3.0 | 3.4 | 5.8 | 1.8 |
| OM isolate downstream | 8.0/6.9 | 5.0 | 2.0 | 5.2 | 2.1 |
| *2012 DOM isolate upstream* | *8.0/6.9* | *5.0* | *3.4* | *5.0* | *1.1* |
| *2012 EfOM isolate* | *8.0/6.9* | *5.0* | *2.5* | *4.6* | *1.4* |
| Pomperaug River (6% (v/v) effluent flow) | | | | | |
| Whole water upstream | 7.4 | 2.4 | 3.6 | 4.3 | 1.2 |
| Whole water downstream | 7.1 | 2.8 | 3.9 | 4.3 | 1.2 |
| OM isolate upstream | 8.0/6.9 | 5.8 | 3.5 | 5.2 | 1.3 |
| OM isolate downstream | 8.0/6.9 | 5.7 | 3.4 | 5.1 | 1.4 |
| *2013 DOM isolate upstream* | *8.0/6.9* | *5.0* | *1.6* | *4.3* | *1.8* |
| *2013 EfOM isolate* | *8.0/6.9* | *5.0* | *0.8* | *6.0* | *2.2* |
| *2012 DOM isolate upstream* | *8.0/6.9* | *5.0* | *1.3* | *4.7* | *1.9* |
| *2011 DOM isolate upstream* | *8.0/6.9* | *5.0* | *1.8* | *3.9* | *1.5* |

[a] DOC (± 0.2 mg $L^{-1}$) for isolates is the prepared concentration in photochemistry experiments, [b] $SUVA_{254}$ ( ± 0.006 L $mg_C^{-1}m^{-1}$), [c] E2/E3 (± 0.07), [d] Fluorescence index (± 0.07), [e] 10 mM phosphate buffer pH for $^3DOM^*$ experiments/ pH for $^1O_2$ experiments.

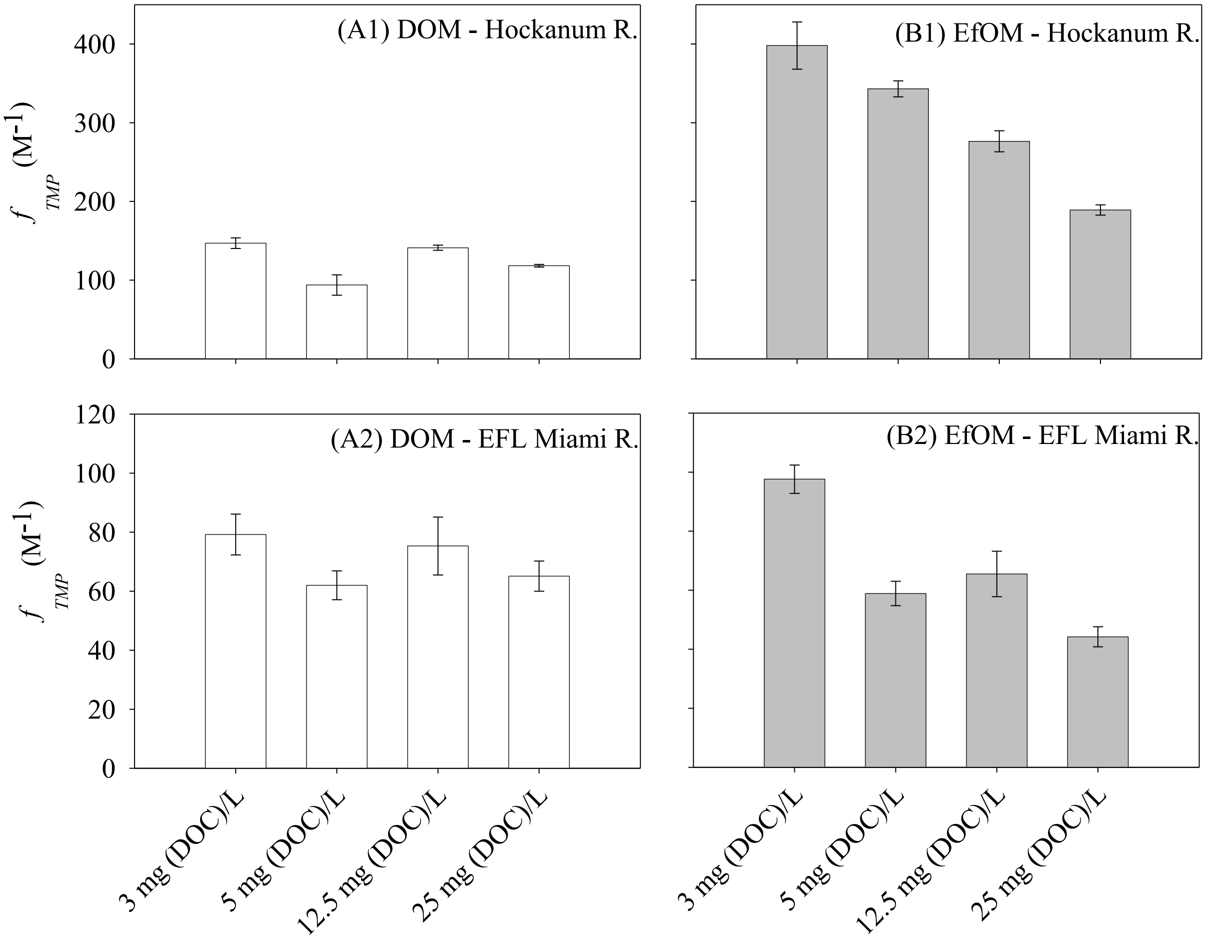

**Figure 1. Influence of dissolved organic carbon (DOC) concentration on triplet quantum yield coefficients ($f_{TMP}$ (M$^{-1}$)) for large-scale OM isolates of Hockanum R. (A1) dissolved organic matter (DOM) and (B1) effluent organic matter (EfOM) and EFL Miami R. (A2) DOM and (B2) EfOM, all collected in 2012.**

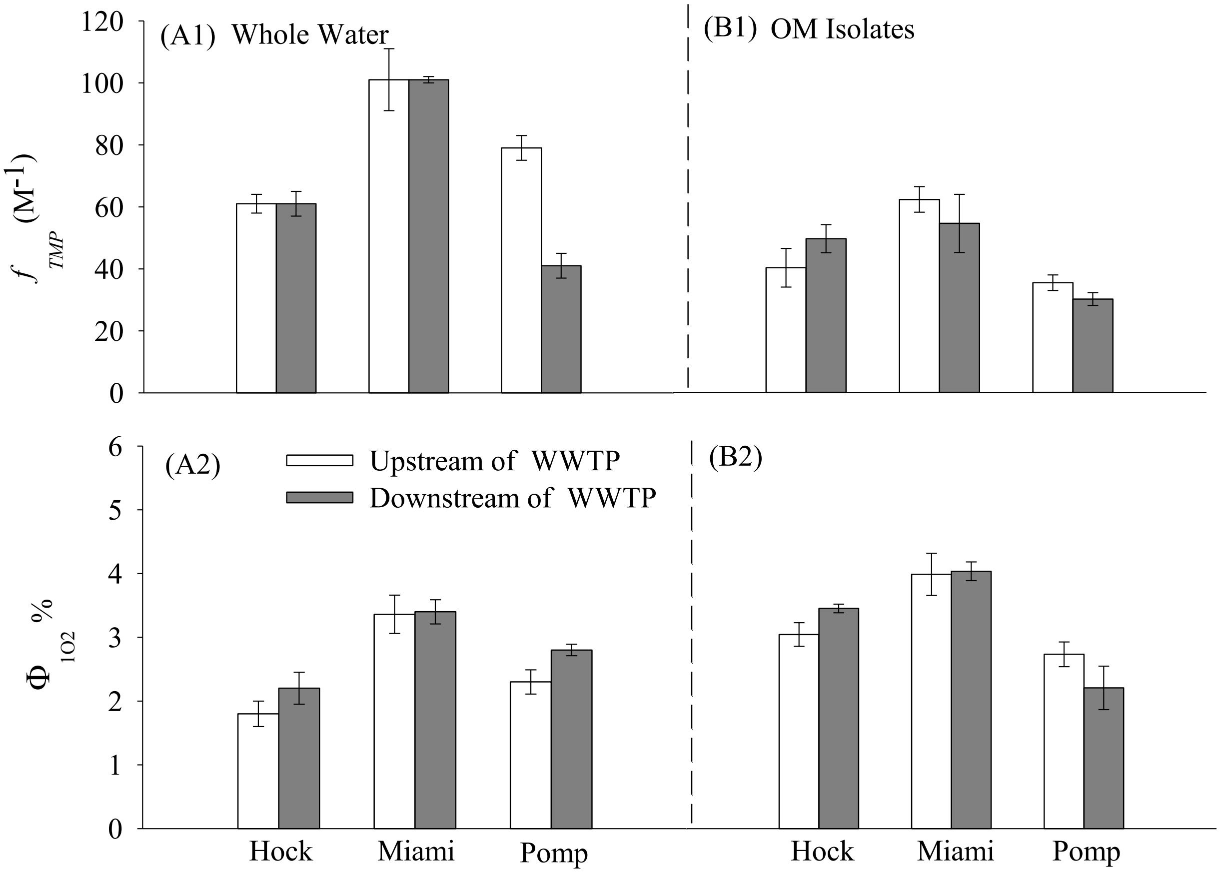

**Figure 2. Triplet quantum yield coefficients ($f_{TMP}$ ($M^{-1}$)) and $^1O_2$ apparent quantum yields ($\Phi_{1O2}$ (%)) for 2013 (A1 & A2) whole water samples and (B1 & B2) OM isolates collected in 2013 at river locations upstream (white bar) and downstream (grey bar) of the WWTP discharges, where Hock = Hockanum R., Miami = EFL Miami R. and Pomp = Pomperaug R.**

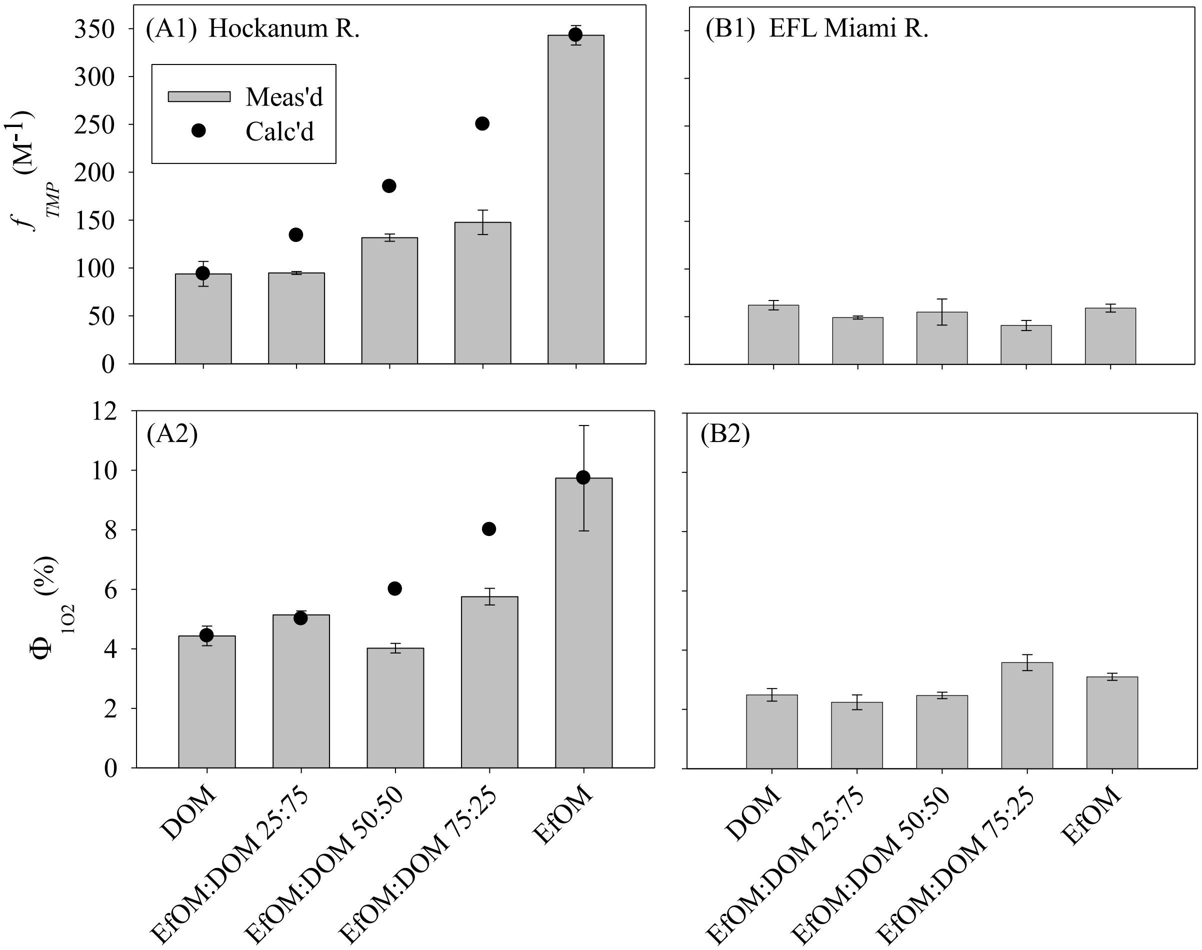

**Figure 3. Triplet quantum yield coefficients ($f_{TMP}$ (M$^{-1}$)) and $^1O_2$ apparent quantum yields ($\Phi_{1O2}$ (%)) for mixtures of large-scale dissolved organic matter (DOM) and effluent organic matter (EfOM) isolates collected in 2012 from (A1 & A2) the Hockanum R and (B1 & B2) the EFL Miami R. Calculated yields assuming conservative mixing (Eq. 1) are shown for the Hockanum R. (●).**

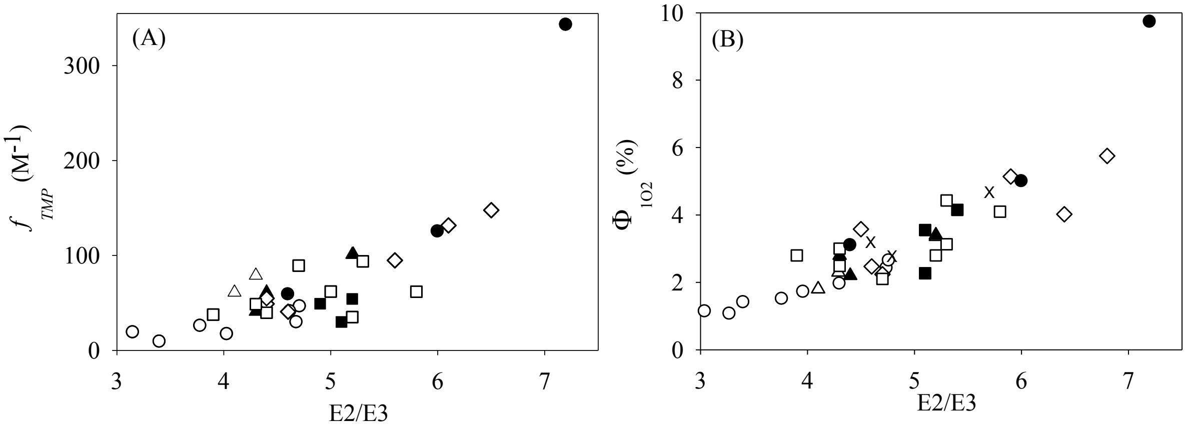

**Figure 4. Trends in sample (A) triplet quantum yield coefficient ($f_{TMP}$ (M$^{-1}$)) and (B) $^1O_2$ apparent quantum yields ($\Phi_{1O2}$ (%)) with DOM optical characteristics and comparison with International Humic Substance Society (IHSS) reference materials: ○ IHSS; □ small/large-scale OM isolates from upstream of the WWTP; ■ small-scale OM isolates from downstream of the WWTP; ● large-scale EfOM isolates; ◊ EfOM:DOM isolate mixtures; Δ whole water from upstream of the WWTP; ▲ whole water from downstream of the WWTP. Previously reported values[25] (×) are shown in B.**

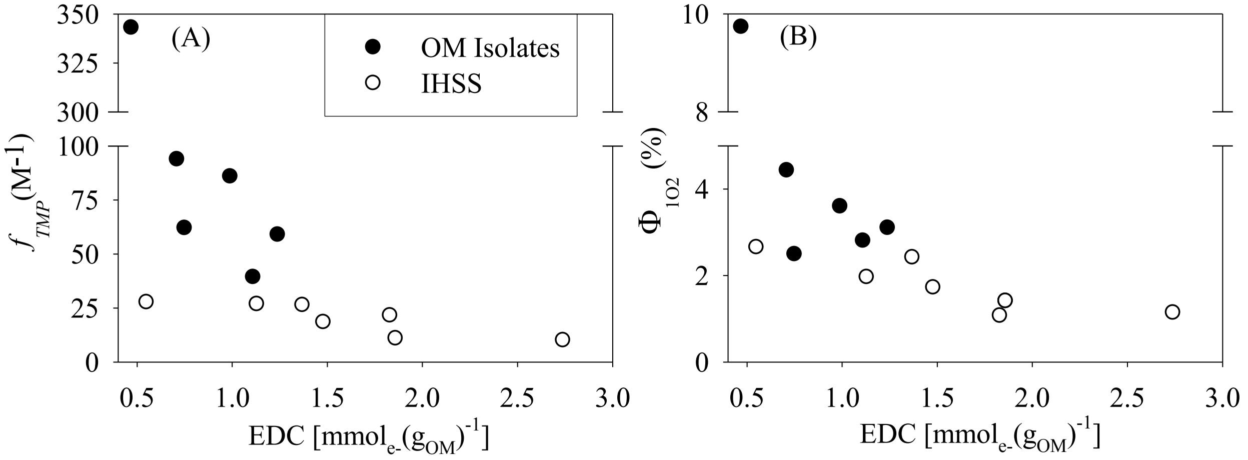

**Figure 5. Relationship between (A) triplet quantum yield coefficients ($f_{TMP}$ (M$^{-1}$)) and (B) $^1O_2$ apparent quantum yields ($\Phi_{1O2}$ (%)) and electron-donating capacities (EDC, pH 7, 0.61 V vs. standard hydrogen electrode) of (●) OM isolates collected in 2012 and comparison with (○) IHSS reference materials obtained from previous work by Aeschbacher et al.[56]**

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