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Molecular Composition and Photochemical Reactivity of Size-Fractionated Dissolved Organic Matter

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Abstract

The photochemical production of reactive species, such as triplet dissolved organic matter (³DOM) and singlet oxygen (¹O₂), contributes to the degradation of aquatic contaminants and is related to an array of DOM structural characteristics, notably molecular weight. In order to relate DOM molecular weight, optical properties, and reactive species production, Suwannee River (SRFA) and Pony Lake fulvic acid (PLFA) isolates are fractionated by sequential ultrafiltration, and the resultant fractions are evaluated in terms of molecular composition and photochemical reactivity. UV–visible measurements of aromaticity increase with molecular weight in both fulvic acids, while PLFA molecular weight fractions are shown to be structurally similar by Fourier-transform ion cyclotron resonance mass spectrometry. In addition, Bray–Curtis dissimilarity analysis of formulas identified in the isolates and their size fractions reveal that SRFA and PLFA have distinct molecular compositions. Quantum yields of ³DOM, measured by electron and energy transfer probes, and ¹O₂ decreased with molecular weight. Decreasing [³DOM]_{ss} with molecular weight is shown to derive from elevated quenching in high molecular weight fractions, rather than increased ³DOM formation. This work has implications for the photochemistry of waters undergoing natural or engineered treatment processes that alter DOM molecular weight, such as photooxidation and biological degradation.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b05140. Additional experimental details, Figures S1–S16, and Tables S1–S6. (PDF)

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Notes

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■ INTRODUCTION

Dissolved organic matter (DOM) is a heterogeneous mixture of biologically derived molecules present in natural and engineered aquatic systems that contributes to environmentally significant processes including carbon cycling,¹ disinfection byproduct formation,² and contaminant fate and transport.³ For example, DOM contributes to the indirect photodegradation of aquatic pollutants, including pesticides⁴ and pharmaceuticals,⁵ through the production of excited triplet states (³DOM) and reactive intermediates, such as hydroxyl radicals.^{6,7} ³DOM are long-lived species resulting from photon absorption by DOM to form excited, singlet DOM and subsequent partial relaxation via intersystem crossing.^{8,9} ³DOM degrades contaminants by direct reaction through energy¹⁰ or electron transfer¹¹ or through the production of reactive species, such as singlet oxygen (¹O₂).¹²

The production of reactive species varies with DOM optical properties,^{13–16} molecular weight (MW),^{14,17} and composition.^{18,19} These structural variations derive from disparities in source (i.e., allochthonous or autochthonous)²⁰ and physical/chemical processing (e.g., irradiation or ozonation).^{21,22} UV–visible spectra of DOM provide insight into the structure and photochemical reactivity of DOM.^{13,15,23} For example, measurements of absorbance ratios, such as $E_2:E_3$ (i.e., the ratio of absorbance at 250 nm to that at 365 nm), and exponential spectral slope terms decrease with molecular weight,^{23–26} while molar absorptivity increases.²⁷ The specific-UV absorbance at 254 nm (SUVA₂₅₄) correlates with DOM aromaticity, as determined by the relative signal intensity in the aromatic region (110–160 ppm) of the ¹³C NMR spectrum, and with molecular weight.^{27–30} Quantum yields of ³DOM^{14,15} and ¹O₂^{13–16,31} are consistently shown to increase with $E_2:E_3$, while reactive species steady-state concentrations generally increase with solution absorbance (i.e., the concentration of dissolved organic carbon; [DOC]).^{17,32}

The environmental activity of DOM is highly influenced by molecular weight, yet accurate measurements of MW are confounded by the structural diversity of DOM and its tendency to form supramolecular assemblies.³³ Analytical techniques applied to estimate DOM MW include diffusivity,³⁴ field flow fractionation,³⁵ fluorescence correlation spectroscopy,³⁴ size exclusion chromatography (SEC),²⁷ reactivity with radical species,³⁶ small-angle X-ray scattering,³⁷ and vapor pressure osmometry.³⁸ Ultrafiltration is also capable of estimating DOM molecular weight and, uniquely, can separate fractions for photochemical experiments without significant dilution.^{32,39} However, MW measurements made with ultrafiltration are

often higher and more variable compared with the above techniques.^{21,34,37,40} Despite an established sensitivity to variation in experimental parameters (e.g., pH and [DOC]),^{37,40} studies using ultrafiltration to fractionate DOM for photochemical experiments typically do not independently evaluate the retention characteristics of their ultrafiltration protocol. Fulvic acid fractions of DOM are generally reported to have average molecular weights near 1 kDa. Autochthonous isolates, such as Pony Lake fulvic acid (PLFA), are typically reported to be lower in molecular weight (186–2400 Da)^{36,41} compared to allochthonous isolates, such as Suwannee River fulvic acid (SRFA; 241–4100 Da).^{27,35,36,38,41}

In DOM fractionated using ultrafiltration, quantum yields of ^3DOM ^{14,42} and $^1\text{O}_2$ ^{31,43} are consistently enhanced in low MW fractions. Conversely, ^3DOM and $^1\text{O}_2$ steady-state concentrations have been observed to increase,³² decrease,^{17,44,45} or remain constant^{31,46} with molecular weight. The lack of correlation between quantum yields and steady-state concentrations is a product of the conflicting trends of increasing absorbance,²⁷ but decreasing quantum yields, with increasing molecular weight.

The application of high-resolution mass spectrometry, such as Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR MS), provides new insight into the molecular composition of DOM.^{29,47–51} FT-ICR MS can identify thousands of individual molecular formulas in a single DOM sample and is used to compare DOM populations with respect to source²⁰ and processing through engineered^{52–54} or environmental systems.^{55–57} FT-ICR MS has been used to demonstrate that lignin-derived formulas are rich in ^3DOM -relevant carbonyls¹⁸ and to identify the classes of DOM that are susceptible to degradation by solar irradiation,²² but it has not been previously used to link DOM structure and ^3DOM photochemistry or to evaluate DOM structural trends with molecular weight.

While an array of correlations between photochemistry, molecular structure, and molecular weight have been observed in DOM, previous efforts have largely combined ultrafiltration protocols using single membranes with structural characterization solely by optical spectroscopy. Alternatively, this research presents a calibrated sequential ultrafiltration protocol with fraction characterization by UV–visible spectroscopy and FT-ICR MS. Further, a range of probes that quantify ^3DOM and $^1\text{O}_2$ production by defined reaction pathways are utilized to resolve changes in DOM photochemical reactivity with molecular weight and to determine the underlying mechanisms of the observed trends between photochemical reactivity and MW.

■ MATERIALS AND METHODS

Materials

SRFA II (2S101F) and PLFA (1R109F) isolates were obtained from the International Humic Substances Society (Denver, CO). Details on other materials and solution preparation are available in the Supporting Information (Section S1).

Ultrafiltration Evaluation

Ultrafiltration was performed with an HP4750 stirred cell (Sterlitech Corp., Kent, WA). The apparatus and protocol are described in detail in Section S2. Retention of six model

compounds (30.0–973.7 Da) by 1 and 3 kDa ultrafiltration membranes was evaluated at pH 3, 7, and 10. Model compound concentrations in stock, permeate, and retentate solutions were determined with UV–visible spectroscopy or high-performance liquid chromatography (HPLC) as described in Section S3. Additionally, the role of [DOC] and pH in determining DOM retention by 1, 3, and 5 kDa ultrafiltration membranes was evaluated with SRFA solutions (Section S2).

Fulvic Acid Isolate Fractionation

PLFA and SRFA solutions (~80 mg-C/L, pH 7) were fractionated with a series of ultrafiltration membranes (3, 5, and 10 kDa) into four nominal molecular weight classes: <3 kDa, 3–5 kDa, 5–10 kDa, and >10 kDa (Section S2). The resultant fractions were evaluated in terms of UV–visible spectroscopy, mass spectrometry, and photochemical analyses, as described below.

DOM Characterization

[DOC] was quantified as non-purgeable organic carbon with a Shimadzu TOC-V analyzer, calibrated against potassium hydrogen phthalate. Solution pH was measured with a Mettler Toledo EL20 pH meter before and after irradiations and typically varied by <0.1 pH units. UV–visible absorbance was determined in 1 nm increments, following dilution to 2 mg-C/L, with a Shimadzu UV-2401PC UV–visible spectrophotometer with quartz cuvettes and a Milli-Q reference cell. All spectra were corrected for blank and long-wavelength (700–800 nm) absorbance. The $E_2:E_3$ and $SUVA_{254}$ ($L\ mg-C^{-1}\ m^{-1}$) values were calculated as described previously.^{23,28} Spectral slope ($S_{275-295}$, nm^{-1}) was calculated with least-squares regression of exponential slopes over 275–295 nm.²³

Mass Spectrometry

FT-ICR MS analysis utilized a 7 T hybrid linear ion trap-FT-ICR MS (Thermo LTQ FT Ultra). Solutions were diluted 1:1 in acetonitrile and introduced into a negative mode electrospray ionization source by direct infusion. Blank solutions consisting of 1:1 Milli-Q:acetonitrile, were prepared and analyzed. Instrument settings are described in Section S4.

Formula Identification and Presentation

Following FT-ICR MS analysis, peak identification was performed by Mash Suite Pro.⁵⁸ Mass-to-charge values were converted to putative masses by three-point internal calibration utilizing commonly identified formulas that were present in both fulvic acids and all molecular weight fractions.⁵⁹ Formulas were assigned to masses only if a second mass 1.003354 ± 0.0001 ($^{13}C - ^{12}C$) greater than the first was also identified and if a possible $^{12}C_{0-\infty} ^{1}H_{0-\infty} ^{16}O_{0-\infty} ^{14}N_{0-1}$ formula was within 0.0001 Da. Formulas were only considered for assignment if they had whole number double bond equivalent values and obeyed the nitrogen rule.

Double bond equivalents (DBE), which describes the number of double bond or ring structure equivalents in a molecule, and aromaticity index (AI), which describes the relative aromaticity of formulas, were calculated for identified formulas.^{22,60} Formulas identified as lignin-like were those with $O:C = 0.29\text{--}0.65$ and $H:C = 0.7\text{--}1.5$.⁶¹ Hierarchical cluster

analysis was performed using Bray–Curtis dissimilarity, which was calculated between the fulvic acid isolates and their molecular weight fractions using R .⁶²

Photochemical Analyses

Prior to irradiations, fulvic acid isolates and their molecular weight fractions were diluted to 2 mg-C/L in air-saturated Milli-Q water and adjusted to $\text{pH } 8 \pm 0.2$ with phosphoric acid and potassium hydrogen phosphate dibasic so that the total added phosphate concentration was 20 mM. *Trans,trans*-2,4-hexadienoic acid (HDA) was added at initial concentrations of 25, 100, 250, 1000, and 2500 μM , while furfuryl alcohol (FFA) and 2,4,6-trimethylphenol (TMP) were added at initial concentrations of 25 μM . Irradiations were conducted in a Rayonet RPR-200 photoreactor containing 16 RPR-3500 bulbs ($\lambda_{\text{max}} = 365 \text{ nm}$, width at half-maximum = 9 nm; Figure S8).⁶³ Total photon flux was calculated by *para*-nitroanisole (PNA)/pyridine actinometry (Section S5).^{64,65} Sample irradiations were conducted for sufficient time as to allow the accurate quantification of HDA isomerization, TMP loss, and FFA loss (10, 60, and 120 min, respectively). Loss of FFA, TMP, and PNA, as well as the isomerization of HDA, were quantified by HPLC (Section S3). Dark controls, prepared as above but not irradiated, and direct photolysis controls, prepared as above without the addition of DOM, were analyzed for each probe compound. No probe reaction was seen in dark controls (results not shown), while direct photolysis loss of FFA and TMP was minimal (Figure S12). Direct isomerization of HDA was observed, but not corrected for, as it accounted for <10% of the observed isomerization in DOM samples (Figure S12).

^3DOM formation rates ($F_{3\text{DOM}}$), quenching rate constants (k_d), steady-state concentrations ($[^3\text{DOM}]_{\text{ss}}$), and quantum yields ($\Phi_{3\text{DOM}}$) quantified with HDA are determined with inverse rate calculations and a previously estimated rate constant for the reaction between ^3DOM and HDA (Section S6).⁶⁶ ^3DOM quantum yield coefficients determined with TMP (f_{TMP})¹⁵ and $^1\text{O}_2$ quantum yields ($\Phi_{1\text{O}_2}$),¹³ as well as steady-state concentrations of ^3DOM , ($[^3\text{DOM}]_{\text{ss,TMP}}$) and $^1\text{O}_2$ ($[^1\text{O}_2]_{\text{ss}}$), measured with TMP and FFA, respectively, are calculated with previously published rate constants (Section S6). Error bars represent the standard deviation of triplicate analyses.

■ RESULTS AND DISCUSSION

Characterization of Ultrafiltration Membranes

Although ultrafiltration is an established technique for the molecular weight fractionation of DOM,^{21,30,67} nominal molecular weight cutoffs (MWCO) overestimate the molecular weight distribution of complex solutions of charged organic molecules.^{34,67,68} Retention of model compounds was observed well below membrane MWCOs (Figure S1a,b) and was a function of molecular weight, molecular charge, and solution pH. For example, formaldehyde (MW = 30.0 g/mol; neutral) was not retained by either membrane under any condition, while 11% and 5% of riboflavin (MW = 376.37 g/mol; neutral at pH 7) was retained by the 1 and 3 kDa membranes at pH 7, respectively (Figure S1a,b). More negatively charged compounds were better retained; for example, retention of tartarazine (465.3 g/mol; charge -3 at pH 7) by a 3 kDa membrane at pH 7 exceeded that of rose bengal (973.7 g/mol; charge -2 at pH 7; 17% vs 15%; Figure S1b). Overall, the retention of small,

charged model compounds was observed far below the specified MWCOs, as seen previously with regenerated cellulose membranes.⁶⁹

The influence of charge interactions on ultrafiltration retention is relevant to DOM due to the presence of ionizable functional groups, such as carboxylates, that will impart negative charges to DOM⁷⁰ and ultrafiltration membranes at environmentally relevant pH values.⁴⁰ The retention of charged model compounds by the ultrafiltration membranes was observed to be a function of solution pH. For example, the retention of tartrazine (charge = -3 at pH 7, -4 at pH 10) by a 3 kDa membrane increased from 17% to 42% when the pH is increased from 7 to 10 (Figure S1b). In contrast, riboflavin (376.36 g/mol; neutral) retention by a 3 kDa membrane only increased from 5% to 7% over the same pH range (Figure S1b).

The model compound results demonstrate the importance of ionizable functional groups and solution pH in the retention of DOM by ultrafiltration. Therefore, we conducted further control experiments to specifically determine the influence of pH and [DOC] on DOM retention. SRFA retention was pH dependent; retention by a 3 kDa membrane increased from $72 \pm 2\%$ to $87 \pm 1\%$ as the pH increased from 3 to 7 (average values over 2.6 to 35.3 mg-C/L; Figure S1c). Carbon concentration was less influential in determining retention, as SRFA retention decreased only slightly from 88% to 85% as [DOC] increased from 2.6 to 35.3 mg-C/L (Figure S1c). Therefore, [DOC] concentrations were not controlled for in the sequential ultrafiltration protocol.

Measurements of the molecular weight of SRFA determined with field flow fractionation (number-averaged: 1150 g/mol; weight-averaged: 1910 g/mol),³⁵ SEC (number-averaged: 1360 g/mol; weight-averaged: 2310 g/mol),²⁷ reactivity with hydroxyl radical (number-averaged: 760 g/mol),³⁶ and vapor pressure osmometry (number-averaged: 829 g/mol)³⁸ report number-averaged molecular weights near 1 kDa and weight-averaged molecular weights near 2 kDa. However, $87 \pm 1\%$ of SRFA DOC (2.6–35.3 mg-C/L) was retained by a 3 kDa membrane, and 22% was retained by a 5 kDa membrane at pH 7 (Figure S1d). This suggests a weight-averaged molecular weight of approximately 3 kDa, higher than estimates utilizing other methods. The observed retention of model compounds and SRFA confirm that MWCOs are not accurate in quantifying the molecular weight of charged compounds.^{34,37} Consequently, the use of nominal MWCOs (i.e., <3 kDa) in describing molecular weight fractions is for sample identification only.

Sequential Fractionation

Ultrafiltration of SRFA and PLFA isolates into <3, 3–5, 5–10, and >10 kDa fractions resulted in quantitative carbon recovery (100% for PLFA, 96% for SRFA; Table 1). The carbon mass balance suggests a lower weight-averaged molecular weight for PLFA than SRFA; 74% of PLFA was captured in the two lower molecular weight fractions, compared with 37% of SRFA (Table 1). This agrees with previous observations of lower MW for PLFA compared to SRFA, as well as lower MW for DOM derived from autochthonous sources compared to allochthonous sources.^{25,35,36,71}

Optical Properties of Fulvic Acid Isolates

UV–visible spectroscopy demonstrates that the sequential ultrafiltration procedure separated the fulvic acid isolates into optically distinct fractions. First, $E_2:E_3$ and $S_{275-295}$ decrease with molecular weight in both isolates (Table 1). These optical properties describe the slope of the absorbance spectra and are inversely proportional to molecular weight.^{23–26} Second, $SUVA_{254}$ increases with MW for both SRFA and PLFA, confirming previous reports of specific UV absorbance and aromaticity increasing with MW in DOM (Table 1).^{27–30} Finally, the rate of light absorption increases with molecular weight both in our photochemical experiments ($R_{a,UV-A}$) and under the solar spectrum ($R_{a,solar}$; Table 1). Collectively, the optical properties demonstrate that higher molecular weight fractions are more aromatic and more colored (i.e., absorb more light in the visible range).

Molecular Composition of Size-Fractionated DOM

Compositional differences between molecular weight fractions were further evaluated with FT-ICR MS. Hundreds of molecular formulas were identified in each fraction, with a higher proportion of N-containing formulas in the PLFA fractions (Table 1), as observed in a previous study of these isolates.⁷² Identified molecular formulas are presented in van Krevelen diagrams (Figures 1 and S6), which allow for qualitative comparison of DOM samples based their molecular composition.^{50,55} For example, SRFA formulas occupy a lower H:C range than PLFA formulas, demonstrating higher aromaticity (Figure 1a,b), in agreement with previous FT-ICR MS analyses of these isolates.^{72,73} The average DBE of formulas identified in bulk SRFA (11.5) agrees with two previous measurements (9.9, 11.4).^{53,74}

The identified molecular formulas in the fractionated isolates demonstrate that the fractions have differing molecular compositions. Increasing aromaticity with molecular weight in SRFA fractions can be observed qualitatively as a decrease in H:C ratios (Figure 1c–f). This trend is confirmed quantitatively as both the fraction of formulas with AI > 0.5 and average DBE increase with molecular weight (Table 1). Conversely, structural trends in PLFA are subtle; each fraction occupies roughly the same region of the van Krevelen diagrams (Figure S6) and slight variations are observed in quantitative measurements (Table 1). The absence of highly aromatic molecules in this microbially derived isolate, confirmed by its low $SUVA_{254}$ values, likely contributes to the compositional similarities between molecular weight fractions.⁷⁵

While compositional trends are apparent between SRFA molecular weight fractions (Table 1 and Figure 1), the number-averaged molecular weight of identified formulas does not vary within fractions of either PLFA or SRFA (Table S4). This result is consistent with a previous study of dialysis-fractionated SRFA²⁹ and derives from factors related to the behavior of DOM in aqueous solution and biases in FT-ICR MS analysis. First, DOM exists in supramolecular assemblies bound together by hydrophobic interactions in aqueous solution.^{33,76} As analysis by FT-ICR MS is preceded by dissolution in acetonitrile and electrospray ionization, the identified formulas are more likely representative of individual DOM molecules than supramolecular assemblies.⁷⁷ Therefore, the ions detected by FT-ICR MS are lower in molecular weight than expected based on the molecular weights determined by

other techniques.⁷⁸ Second, the probability that a molecule will be both ionized by electrospray and detected by FT-ICR MS is related to its molecular weight and FT-ICR MS parameters.^{78,79} Therefore, each FT-ICR MS analysis will selectively favor molecules within a certain molecular weight range, as is apparent in the wide variation of mass spectra reported for SRFA.^{29,49,79–82} Consequently, results obtained with FT-ICR MS accurately reflect trends in DOM composition, but are not useful in determining DOM average molecular weights.

Some formulas appear in the van Krevelen diagrams of molecular weight fractionations but not in the corresponding bulk fulvic acid (Figures 1 and S6). This indicates their relative enrichment and improved ionization in the molecular weight fraction, rather than their creation by the fractionation procedure. Similar observations were made for SRFA that was fractionated by dialysis and analyzed by Orbitrap MS.²⁹

Bray–Curtis dissimilarity was calculated to determine the relatedness of molecular weight fractions in terms of their shared formulas (Figure S7).^{55,74} The highest dissimilarity was observed between the SRFA-derived and PLFA-derived fractions, in agreement with our understanding of them as distinctive source end-members (i.e., derived from allochthonous and autochthonous sources). PLFA is shown to be most similar to its 3–5 kDa molecular weight fraction, while SRFA is most similar to its 5–10 kDa fraction. This suggests that PLFA has a lower average molecular weight than SRFA, in agreement with comparisons based on radical reactivity and SEC.^{36,71}

Although there are similarities between the FT-ICR MS data and optical spectroscopy results, observed trends with molecular weight between the two approaches do not always agree. UV–visible spectroscopy measurements (i.e., $E_2:E_3$, $S_{275-295}$, $SUVA_{254}$) show more similarity between the bulk fulvic acid isolates and their higher molecular weight fractions (Table 1), while Bray–Curtis dissimilarity analysis of formulas identified using FT-ICR MS suggests the bulk fulvic acids, especially PLFA, are more similar to the lower molecular weight fractions (Figure S7). These differences could be due to limitations of the respective techniques; UV–visible spectroscopy only detects molecules that absorb light, while FT-ICR MS only detects molecules that are ionized using the selected ionization method. Therefore, it is likely that divergent subpopulations of DOM are sampled by each measurement technique, resulting in different apparent molecular weight distributions.

Despite the possibility that different subpopulations are measured by UV–visible spectroscopy and FT-ICR MS, in some instances, the two techniques provide complementary structural information. Two FT-ICR MS aromaticity measurements, average DBE and the fraction of formulas with $AI > 0.5$, increase linearly with $SUVA_{254}$ in SRFA fractions but show little relationship in PLFA fractions due to the lack of aromatic molecules in this isolate (Figure 2). While a previous work demonstrated a correlation between $SUVA_{254}$ and ^{13}C NMR aromaticity measurements,²⁸ we report the first comparison of aromaticity measurements by UV–visible spectroscopy and FT-ICR MS. The agreement of distinct measurement techniques is further confirmation that aromaticity increases with DOM molecular weight in allochthonous DOM isolates.

Photochemistry of Size-Fractionated DOM

We examined the photochemical production of reactive species by bulk and size-fractionated fulvic acid isolates using species-specific probe compounds. Here, $\Phi_{3\text{DOM}}$ in SRFA (0.0119 ± 0.0001) measured with HDA agrees with a previous measurement in Suwannee River natural organic matter (SRNOM; 0.012),⁶⁶ while f_{TMP} is higher than previously reported in SRFA ($63 \pm 2 \text{ M}^{-1}$ vs $14.4\text{--}25.7 \text{ M}^{-1}$).^{14,83,84} Also, $\Phi_{1\text{O}_2}$ measured in bulk SRFA (0.015 ± 0.001) and PLFA (0.019 ± 0.002) are within the range of previously reported values for SRFA ($0.0138\text{--}0.0211$)^{8,31,85} and PLFA ($0.0134\text{--}0.0204$).^{31,85} Notably, all three quantum yields are higher in PLFA than SRFA (Figure 3), agreeing with a previous measurement of $\Phi_{1\text{O}_2}$ in the two fulvic acid isolates.⁸⁶

In ultrafiltration-fractionated fulvic acid isolates, $\Phi_{3\text{DOM}}$ and f_{TMP} increase with $E_2:E_3$ (Figure 3a,b), confirming that the efficiency of ^3DOM production decreases with molecular weight. Additionally, $\Phi_{1\text{O}_2}$, an indirect measure of ^3DOM production,⁸ also increases with $E_2:E_3$ (Figure 3c). Trends in quantum yields are reported with respect to $E_2:E_3$, which is inversely related to molecular weight, to match the approach of previous studies and avoid reliance on nominal MWCs.^{13,14,16,31} As observed with the bulk isolates, $\Phi_{3\text{DOM}}$ and f_{TMP} are generally higher in PLFA than SRFA within corresponding molecular weight fractions (Table S5). However, the slopes of $\Phi_{3\text{DOM}}$ with $E_2:E_3$ are similar among PLFA and SRFA fractions, while the slope of f_{TMP} with $E_2:E_3$ in PLFA fractions is twice that in SRFA fractions (Table S6). Plots of $\Phi_{1\text{O}_2}$ with $E_2:E_3$ show more scatter than the triplet quantum yield slopes, and the slope in SRFA fractions is almost twice that in PLFA fractions (Figure 3c, Table S6).

An inverse relationship between quantum yield and molecular weight has been reported in size-fractionated DOM and in comparisons of diverse natural DOM samples. For example, triplet quantum yields were higher in the fulvic acid fraction that passed through a 1 kDa ultrafiltration membrane than the fraction retained by 30 kDa ultrafiltration membrane,⁴² and f_{TMP} decreased with increasing molecular weight in SRFA fractionated by a 5 kDa ultrafiltration membrane.¹⁴ Similarly, inverse relationships between $\Phi_{1\text{O}_2}$ and apparent MW in ultrafiltration-fractionated DOM samples have been observed in wastewater effluent,³¹ Mississippi River DOM,⁴³ and SRFA.¹⁴ The relationship between ^3DOM production efficiency and molecular weight has also been expressed as linear regressions of $^1\text{O}_2$ quantum yields with $E_2:E_3$ and slopes reported here agree with published results. Comparisons of diverse DOM samples have reported slopes of $\Phi_{1\text{O}_2}$ with $E_2:E_3$ ranging from 0.00196 to 0.0159^{13,14,16,31} that bound our observed overall slope for both SRFA and PLFA ($\Phi_{1\text{O}_2} = 0.0137 \times E_2:E_3 - 0.0455$). Slopes of $\Phi_{3\text{DOM}}$ and f_{TMP} with $E_2:E_3$ in ultrafiltration-fractionated DOM samples have not been previously reported.

$^1\text{O}_2$ is formed from reaction between O_2 and ^3DOM ,¹² and slopes of $\Phi_{3\text{DOM}}$ and $\Phi_{1\text{O}_2}$ with $E_2:E_3$ are similar in fractions of PLFA and SRFA (Table S6). However, $\Phi_{1\text{O}_2}$ is greater than $\Phi_{3\text{DOM}}$ in most molecular weight fractions (Figure 3a,c, Table S5). These results may be attributable to differences in the ^3DOM populations capable of reaction with HDA and O_2 . While both HDA and O_2 quench ^3DOM by an energy transfer mechanism,¹² ^3DOM exists in a range of triplet energies,⁸⁷ and while the triplet energy of HDA ($\sim 250 \text{ kJ/mol}$)⁸⁸ is greater than the reported average triplet energy of fulvic acids ($\sim 175 \text{ kJ/mol}$),⁴² the singlet energy of

O₂ (94 kJ/mol)⁸⁹ is lower. Therefore, it is likely that a larger fraction of the overall ³DOM populations is susceptible to quenching by O₂ (i.e., resulting in ¹O₂ formation) than HDA.

Here, f_{TMP} , and other measurements with TMP, potentially probe a third category of ³DOM that is defined by reaction through an electron transfer mechanism. As TMP has a one-electron oxidation potential of 1.22 V, it reacts by electron transfer with ³DOM that have excited state reduction potentials greater than that value.⁸⁷ It is possible that ³DOM with excited state reduction potentials less than 1.22 V may oxidize TMP through proton-coupled electron transfer (PCET).⁸⁴ If TMP oxidation were occurring by PCET, TMP oxidation rates would decrease if the alcohol proton on TMP were replaced with deuterium. However, when PLFA molecular weight fractions were irradiated in 3:1 D₂O:H₂O, no change in f_{TMP} was observed ($f_{\text{TMP,D2O}}/f_{\text{TMP,H2O}} = 0.96 \pm 0.08$, Table S5, Figure S14). This suggests that PCET was not occurring and implies that the majority of ³DOM that oxidize TMP in all PLFA molecular weight fractions have one-electron reduction potentials above 1.22 V.

Steady-state concentrations are relevant for predictions of contaminant fate. [³DOM]_{ss} averaged 1×10^{-14} and 7×10^{-14} M over all bulk and molecular weight fractions when measured with HDA and TMP, respectively (Figures 4a and S15a, Table S5). [¹O₂]_{ss} was an order of magnitude higher (average $= 1.9 \times 10^{-13}$ M) than [³DOM]_{ss} measured by HDA (Figure S15b, Table S5). Similar comparisons using FFA and HDA in Everglades water⁹⁰ and prairie pothole water⁹¹ have reported [¹O₂]_{ss} values that are 1 to 2 orders of magnitude higher than [³DOM]_{ss}. While [¹O₂]_{ss} may exceed [³DOM]_{ss} in oxygenated waters due to differences in their respective solution quenching rates, it is unlikely that [¹O₂]_{ss} will be more than a factor of 2 higher than [³DOM]_{ss}.^{8,12,87} The remaining difference in steady-state concentrations may be due to the higher selectivity of HDA than O₂ as a ³DOM quencher, as discussed above.

We observed higher [³DOM]_{ss} by HDA in bulk PLFA than SRFA (Figure 4a), in agreement with a previous comparison of the same fulvic acids using the same ³DOM probe.⁹¹ [¹O₂]_{ss} was also higher in PLFA than SRFA, while [³DOM]_{ss} measured by TMP was roughly equal in the fulvic acids (Figure S15, Table S5). [³DOM]_{ss} measured with HDA increased linearly with $E_2:E_3$ in both isolates, while [³DOM]_{ss,TMP} and [¹O₂]_{ss} do not show significant relationships (Figures 4a and S15). [³DOM]_{ss} and [¹O₂]_{ss} have been reported to increase,³² decrease,^{17,44,45} or show no trend with molecular weight,^{31,46} as the elevated quantum yields of low molecular weight DOM is typically offset by lower rates of light absorbance. The divergence in trends of steady-state concentrations with molecular weight may reflect differences in the sampled ³DOM populations or be a reflection of higher variability in analyses conducted with a single concentration of TMP and FFA.

Mechanisms Resulting in Photochemical Trends

Here, Φ_{3DOM} is determined as a function of the rates of ³DOM formation and light absorbance:⁶⁶

$$\Phi_{3\text{DOM}} = \frac{F_{3\text{DOM}}}{R_{\text{a,UV-A}}} \quad (1)$$

The initial HDA isomerization rate was quantified under a range of initial probe concentrations within each molecular weight fraction. Assuming that physical quenching of ^3DOM by HDA is insignificant compared with chemical quenching, this technique allows for the calculation of ^3DOM formation rates and quantum yields in each fraction without the use of estimated rate constants (eq S6).⁶⁶ Within the fractions of each isolate, ^3DOM formation rates are consistent with respect to $E_2:E_3$, except for the SRFA >10 kDa fraction ($E_2:E_3 = 4.04$), which reports lower formation rates than the other SRFA fractions (Figure 4b). When this data point is removed, the p-value of the linear regression between $F_{3\text{DOM}}$ and $E_2:E_3$ in SRFA fractions increases from 0.08 to 0.43, demonstrating the weakness of the correlation. Conversely, $R_{\text{a,UV-A}}$ strongly decreases with $E_2:E_3$ (Figure 4c), suggesting that a structural trait of larger DOM results in increased absorbance. Therefore, the lower ^3DOM quantum yields of the high MW fractions are due to increased light absorbance, likely from chromophores that do not take part in ^3DOM production, rather than decreased production of ^3DOM .

Similarly, ^3DOM steady-state concentrations with HDA are determined as the ratio of ^3DOM formation rates and quenching rate constants:⁹²

$$[^3\text{DOM}]_{\text{ss}} = \frac{F_{3\text{DOM}}}{k_{\text{d}}} \quad (2)$$

As discussed above, $F_{3\text{DOM}}$ are essentially constant between the molecular weight fractions of each isolate. However, k_{d} decreases linearly with $E_2:E_3$ in both isolates (Figure 4d). Therefore, the decreased $[^3\text{DOM}]_{\text{ss}}$ observed in high molecular weight fractions is likely attributable to increased quenching of ^3DOM , rather than decreased formation of ^3DOM . However, it should be noted that k_{d} measurements rely on estimated rate constants for reaction between ^3DOM and $t,t\text{-HDA}$ (k_{p}), and that variations in this rate constant could influence apparent trends. The quenching rate constants reported here ($2.2\text{--}7.3 \times 10^6 \text{ s}^{-1}$) are similar to those determined in SRNOM by a similar method ($2.5\text{--}3 \times 10^6 \text{ s}^{-1}$)⁶⁶ but higher than quenching rates determined by measurements of $^1\text{O}_2$ formation rates and TMP loss rates ($5\text{--}50 \times 10^4 \text{ s}^{-1}$).^{8,93} While inaccuracies in the estimated value of k_{p} introduce some uncertainty, these results suggest shorter ^3DOM lifetimes for higher energy ^3DOM that is capable of reaction with $t,t\text{-HDA}$.

The above observations agree with a model of ^3DOM photochemistry in which ^3DOM formation is related to the concentration of specific functional groups, such as borohydride-reducible carbonyls,⁹³ while light absorption is controlled by intramolecular charge-transfer interactions.^{94,95} In molecular weight fractionated isolates, ^3DOM formation rates correlate with the percentage of lignin-like formulas (Figure S16a), which have previously been identified as high in concentration of borohydride-reducible carbonyl groups.¹⁸ However,

the lack of correlation between $\Phi_{3\text{DOM}}$ and the percentage of lignin-like formulas (Figure S16b), coupled with the strong correlations between molecular weight and optical properties, such as $E_2:E_3$ and the rate of light absorbance (Table 1), agree with the assertion that DOM light absorbance and photochemical efficiency are largely determined by the mutual accessibility of charge-transfer partners.^{94,95}

Environmental Implications

The molecular weight of DOM decreases as natural waters flow from uplands to the open ocean due to the combined action of bio- and photodegradation.^{23,83,96} Similarly, water treatment processes, such as UV-irradiation and ozonation, lower the average MW of DOM.²¹ We observed increased light absorbance with MW in both isolates, which suggests processes that lower the MW of DOM will deepen the photic zone and enhance direct contaminant photodegradation, regardless of DOM source or other co-occurring structural trends.

Additionally, we demonstrate that structural trends with molecular weight are not necessarily shared between DOM samples from divergent sources and that resolving these trends may require specific analytical methods or approaches. For example, SRFA aromaticity increases with molecular weight by both UV-visible spectroscopy and FT-ICR MS. The same trend is observed in PLFA using UV-visible spectroscopy, while FT-ICR MS analyses suggest that the PLFA molecular weight fractions are structurally similar. Accordingly, the use of only UV-visible spectroscopy or FT-ICR MS to evaluate DOM structural modifications may result in the determination of trends that would be contradicted by other analytical methods.

While ^3DOM and $^1\text{O}_2$ quantum yields increase with $E_2:E_3$, $[^3\text{DOM}]_{\text{ss}}$, which determines the rate of indirect contaminant photodegradation, increases with $E_2:E_3$ when measured by HDA, but not TMP, at a constant [DOC]. This demonstrates that the mechanism by which a contaminant reacts with ^3DOM may influence how indirect photolysis rates change with DOM molecular weight. Further, photochemical studies that rely on a single probe compound to quantify ^3DOM production among diverse DOM samples may overlook trends in photoreactivity. This has been observed in natural systems as a previous study of DOM photochemistry identified divergent trends in $^1\text{O}_2$ and ^3DOM production as DOM moved through an estuary.⁹⁰ Our observation that ^3DOM formation rates are constant with molecular weight suggests that environmental or engineered processes that modify molecular weight may not alter the carbon-normalized ^3DOM formation rates. However, the decrease in light absorbance that accompanies decreasing DOM molecular weight may allow indirect photodegradation to occur deeper in the water column and increase the overall contaminant loss rate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. van Krevelen diagrams for all identified DOM molecular formulas in the bulk (a) PLFA and (b) SRFA fulvic acid isolates, as well as (c) the <3 kDa, (d) 3–5 kDa, (e) 5–10 kDa, and (f) >10 kDa fractions of SRFA.

Figure 2.

(a) Average number of double bond equivalents in FT-ICR MS identified formulas against $SUVA_{254}$ and (b) fraction of FT-ICR MS identified formulas with $AI > 0.5$ against $SUVA_{254}$. Solid lines denote linear regressions of bulk fulvic acid isolates (solid markers) and molecular weight fractions (hollow markers) for SRFA (squares) and PLFA (circles). Dotted lines denote 95% confidence intervals of the linear regressions.

Figure 3.

(a) Quantum yield of 3DOM quantified using HDA, (b) quantum yield coefficient f_{TMP} , and (c) quantum yield of 1O_2 against $E_2:E_3$ in bulk fulvic acid isolates (solid markers) and molecular weight fractions (hollow markers). Dashed lines indicate linear regressions of all samples from each isolate.

Figure 4.

(a) Steady-state concentration of ^3DOM , (b) formation rate of ^3DOM , and (d) solution quenching rate of ^3DOM against $E_2:E_3$, all measured with HDA. (c) Rates of light absorbance ($R_{a,UV-A}$) of bulk fulvic acid isolates and molecular weight fractions at the start of photolysis experiments as a function of $E_2:E_3$. Dashed lines indicate linear regressions within each isolate, with bulk fulvic acid isolates denoted with solid markers and molecular weight fractions denoted with hollow markers.

Table 1

Organic Carbon, UV-Visible Spectroscopy, and FT-ICR MS Characterization of Fulvic Acid Isolates and Their Molecular Weight Fractions^a

carbon		UV-visible spectroscopy				FT-ICR MS			
mass balance (%)	$E_2:E_3$	SUVA ₂₅₄ (L mg ⁻¹ C ⁻¹ m ⁻¹)	$S_{275-295}$ (nm ⁻¹)	$R_{a,UV-A}$ (10 ⁻⁸ E cm ⁻³ s ⁻¹)	$R_{a,solar}$ (10 ⁻¹⁰ E cm ⁻³ s ⁻¹)	identified formulas (% CHON)	average DBE	AI > 0.5 (%)	lignin-like (%)
Pony Lake fulvic acid									
bulk	(100)	4.74	3.32	0.0123	5.79	10.6	7.9	4.2	33
<3	39	5.86	2.66	0.0132	3.67	6.48	7.5	5.0	31
3-5	35	5.31	2.37	0.0129	3.63	6.02	7.9	5.5	29
5-10	22	4.58	3.38	0.0125	6.07	10.5	8.0	7.9	29
>10	4	3.79	5.12	0.0114	10.8	19.9	8.0	8.3	35
Suwannee River fulvic acid									
bulk	(96)	4.58	4.83	0.0113	8.26	13.9	11.5	39	42
<3	25	5.30	3.80	0.0118	5.61	9.38	10.3	26	46
3-5	12	5.06	3.68	0.0126	5.68	9.27	10.7	32	46
5-10	39	4.66	4.96	0.0114	8.28	13.7	12.2	48	34
>10	19	3.91	5.93	0.0106	11.8	20.6	13.8	69	19

^aThe overall carbon mass balances of fractionation procedures are included in parentheses. Rates of light absorbance are calculated based on the irradiance of the UV-A light source ($R_{a,UV-A}$) used in photochemistry experiments, as well under modeled sunlight ($R_{a,solar}$).