Supporting Information

What is the Molecular Weight of "High" Molecular Weight Dissolved Organic Matter?

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Summary

8 pages, 3 figures, 2 tables

HMWDOM Sampling Location.

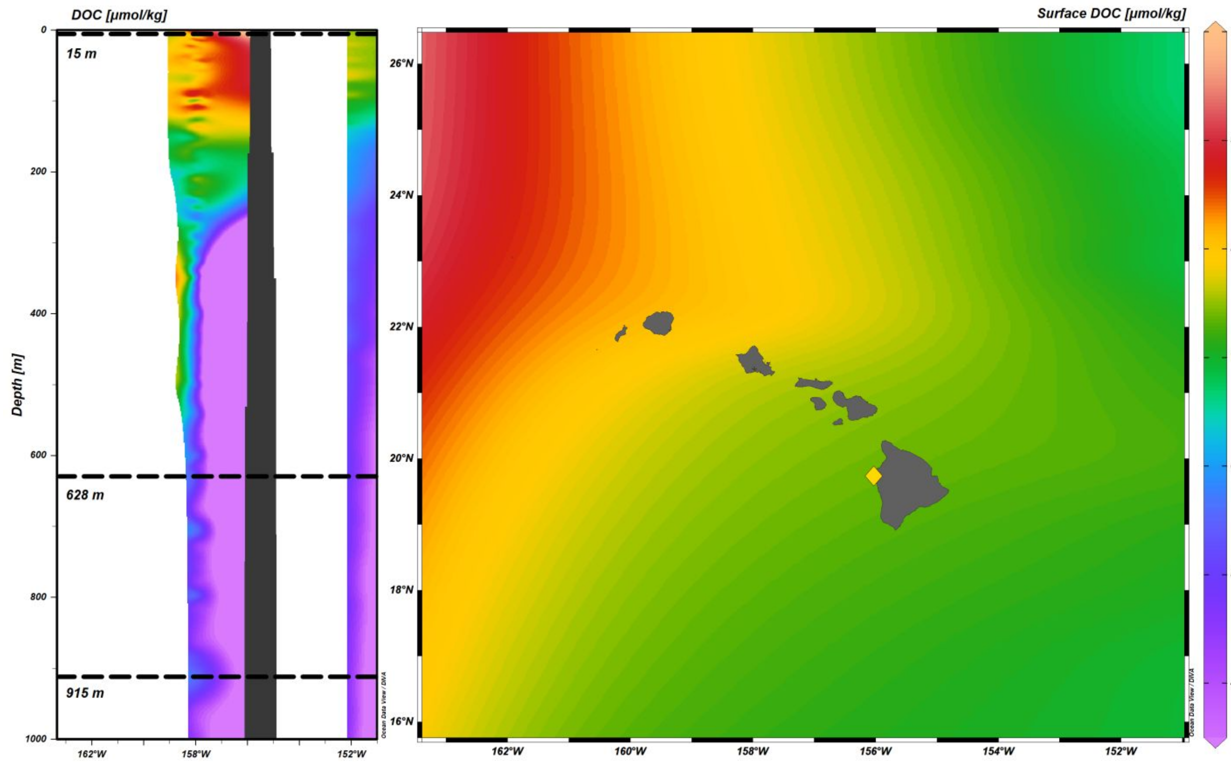


Figure S1. Map of the Hawaiian Islands (plotted with Ocean Data View[1]) showing DOC concentrations in the surface ocean and a profile of DOC concentrations through the first 1000 m of the water column. The yellow diamond indicates the location of the NELHA site, and the dashed lines indicate the sampling depths. DOC data were taken from Hansell et al[2] and from the Hawaii Ocean Time-series.[3]

Calculating the Concentrations of Humic Substances in HMWDOM Samples.

To calculate the concentration of the components of HMWDOM, a ^{1}H NMR spectrum of purified APS was subtracted from each HMWDOM spectrum, yielding an HS difference spectrum. The area of the HS difference spectrum was determined as a percent of the total spectral area of the sample, yielding the percent of protons attached to HS compounds. This value was then converted to a percent of carbon atoms in HS using a ratio of using 1:1.4 carbon to protons, which was based on the stoichiometry of HS chemical formula determined by high-resolution mass spectrometry performed in positive mode and on ^{13}C NMR integration data. The identified formulas had a number averaged molecular weight (MW) of 372 Da, much lower than the average MW of HS calculated from DOSY but had similar spectroscopic properties to HS.[4] Once the relative amount of carbon in HS was calculated, the total concentration of HS was determined from the total DOC concentration measured at each sample depth (75 µmol kg^{-1} and 39 µmol kg^{-1} for the 15 m and 915 m samples, respectively) , and the fraction of DOC contained in HMWDOM (15%).

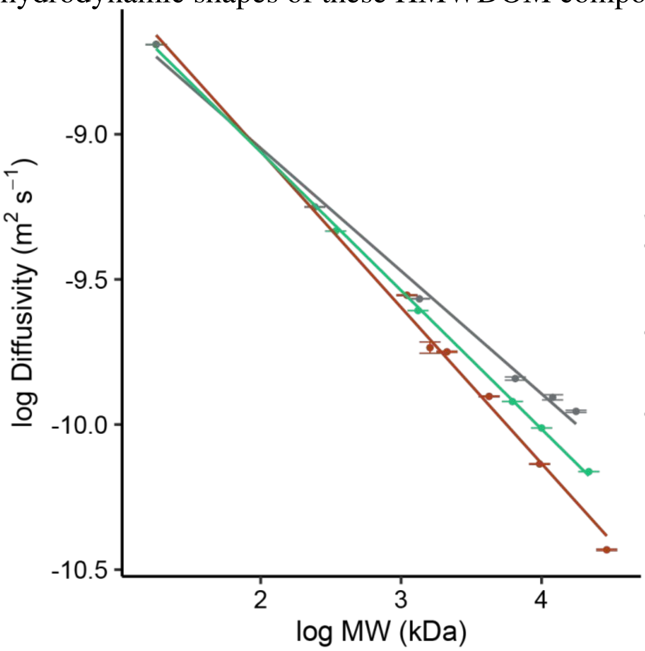
DOSY Pulse Sequence Parameters.

Pulse Sequence	stebpgp1s19
Gradient Steps	32
Size of FID (TD)	F2: 16384 F1: 32
Number of Scans (NS)	256
Dummy Scans (DS)	4
Spectral Width (SW)	F2: 12.1319 ppm F1: 9.9174 ppm
Transmitter Offset (O1P)	4.701 ppm
Dwell Time (DW)	103 μsec
Pre-scan Delay (DE)	10 μsec
Relaxation Delay (D1)	2 sec
Delay for Gradient Recovery (D16)	0.2 msec
Delay for Water Suppression (D19)	0.15 msec
Diffusion Time (Δ, D20)	0.2 sec
Gradient Pulse Name 1 (GPNAM 1)	SMSQ10.100
Gradient Pulse Z Strength 1 (GPZ1)	-20%
Gradient Pulse Name 6 (GPNAM 6)	SMSQ10.100
Gradient Pulse Z Strength 6 (GPZ6)	100%
Spoil Gradient Name (GPNAM 7)	SMSQ10.100
Spoil Gradient Pulse Z Strength (GPZ7)	-17.3%
Watergate Pulse Power (P16)	1000 μsec
Spoil Gradient Pulse Power (P19)	600 μsec
Gradient Pulse Power (δ/2, P30)	1150 μsec

Molecular Weight Calibration of DOSY-Derived Diffusivity Measurements.

The relationship between diffusivity and MW of a molecule depends on its shape. Molecules that have a primary axis (rods) have a lower diffusivity for a given MW, while molecules that are more spherical (balls) have a higher diffusivity for a given MW.[5,6] Using suites of MW standards that have different shapes, the diffusivity–MW relationship over the range of molecular shapes can be constrained.[7–10] Polystyrene sulfonate displays rod-like characteristics and has been used previously as a MW standard for natural humic acid samples, which take on a flexible rod-like conformation at low concentrations and pH greater than 3.5.[11–19] The diffusivities of globular protein standards were measured as representative of spherical, ball-like molecular shapes. The APS component was modeled by pullulan, a linear polysaccharide made up of α-(1→6)-α-(1→4)-maltotriose units, that has an intermediate shape between rods and balls.[20–23] The log(diffusivity) vs log(MW) relationship was determined for three suites of MW standards.

Regressions of these relationships were highly linear ($r^2 > 0.99$) across the diffusivity range of HMWDOM (Figure S1). The slopes of the regressions agree well with previous studies.[20,24,25] For the rest of this discussion, the MW of APS and HS will be calculated using DOSY standard curves of pullulan and polystyrene, respectively, as the most appropriate representation of the hydrodynamic shapes of these HMWDOM components.



Molecular Weight Standard	Slope	Y-Intercept	r^2
Polystyrene Sulfonate	-0.538 (±0.018)	-7.98 (±0.061)	0.99
Pullulan	-0.477 (±0.006)	-8.11 (±0.020)	0.99
Globular Proteins	-0.423 (±0.017)	-8.20 (±0.058)	0.99

Figure S2. Relationships between measured diffusivities and polystyrene sulfonate (red), pullulan (green), and globular protein (grey) MW standards. Error bars denote the uncertainty in the diffusivity calculation. Linear standard curves were calculated using ordinary least-squares regression models. The table lists the model outputs with standard errors. The data show a strong linear relationship between log(diffusivity) vs log(MW) across the entire MW range examined. The spherical globular proteins displayed a greater diffusion coefficient for a given molecular weight, compared to the linear polystyrene molecules, demonstrating the effect of molecular shape on diffusivity.

Validation of the DOSY Technique.

Molecular weights of HMWDOM components were determined by measuring their diffusivity in D_2O and converting to MW using calibrations of pullulan and sulfonated polystyrene of known MWs. In addition to molecular conformation, several factors influence the measured diffusivity of a molecule, including the viscosity of the solution and molecular association/aggregation.[26] D_2O was used as the solvent for all samples, and variations in solution viscosity were minimized by using a low and consistent sample concentration of 1 mg mL^{-1} throughout the analysis. The effects of molecular aggregation were explored by measuring diffusivity of the 15 m HMWDOM samples in different solvents. Diffusivity was also measured under three pH conditions (4, 7, and 10) and three salinities representing 50%, 100%, and 125% of the average seawater salinity of 35 (Figure S1). Finally, diffusivities were measured in methanol and in a 10 mM EDTA solution to promote disaggregation.[27] No difference in the calculated MWs of APS or HS was detected in any treatment, suggesting that under the conditions used in this study, neither molecular aggregation nor viscosity had a significant impact on the apparent MW of the HMWDOM samples in aqueous solution. Therefore, the diffusion measurements provide a good approximation of MW. These results are supported by previous studies, which observed minimal aggregation of HS at concentrations below 4 mg mL^{-1}.[7,16]

Table S2. Effects of solvent conditions on DOSY-derived diffusivity. Standard conditions for samples were D_2O with 0 salinity at pH = 7. Percent deviation denotes the difference in the diffusivity measured under different solvent conditions compared to the standard conditions. No significant deviation was detected for any of the solvent conditions ($\alpha = 0.05$).

Solvent Parameter		%Deviation	%RSD
Salinity			
	17	0.0%	8.9%
	35	1.1%	9.8%
	44	2.3%	10%
pH			
	4	6.5%	20%
	10	4.7%	20%
Solvent			
	Methanol	12%	49%
	Water + EDTA	6.7%	21%

Span Factor Calculation.

A span factor is a unitless metric that describes the broadness of a distribution. MW distributions of the components of HMWDOM were obtained by taking a vertical slice of the DOSY spectrum in areas attributed primarily to one component (3.30 ppm for APS and 2.20 ppm for HS). To compute the span factor of the MW distributions of the components of HMWDOM, the cumulative frequency curve of the distribution was first calculated. From this curve, the 10th, 50th, and 90th percentiles (D_{v10}, D_{v50}, and D_{v90}, respectively) MWs of the distribution can be directly determined as the points on the cumulative curve which are 10%, 50%, and 90% of the maximum height of the curve (Figure S2). Span is computed as $(D_{v90}-D_{v10})/D_{v50}$.

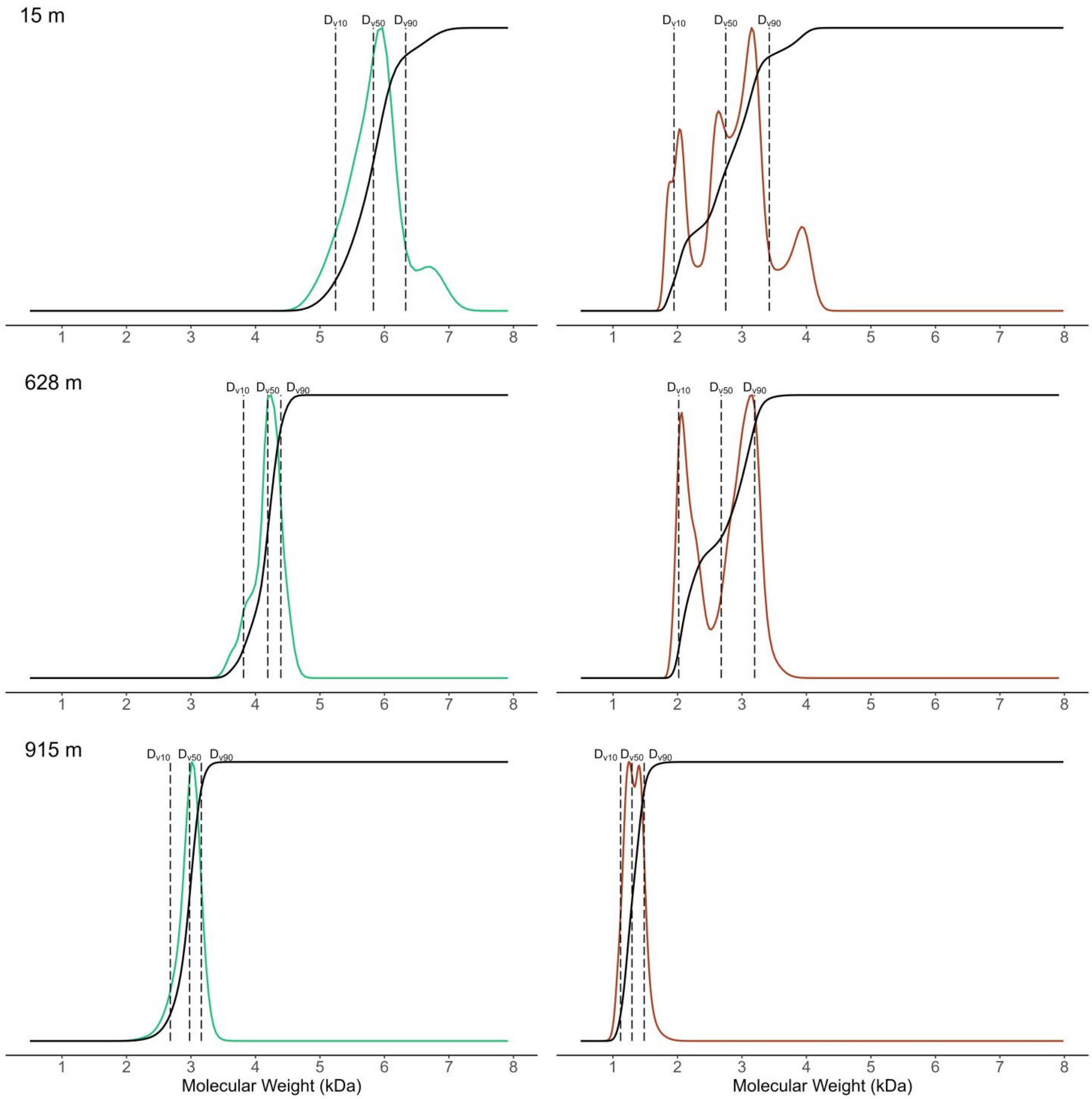


Figure S3. The molecular weight distribution of the APS (green) and HS (orange) components of HMWDOM. The black solid line denotes the cumulative frequency curve of the distribution. To compute the span factor, the 10th, 50th, and 90th percentiles (D_{v10}, D_{v50}, and D_{v90}, respectively) of the MW distribution (dashed vertical lines) were calculated.

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