High field NMR Spectroscopy and FTICR Mass Spectrometry: Powerful Discovery Tools for the Molecular Level Characterization of Marine Dissolved Organic Matter from the South Atlantic Ocean

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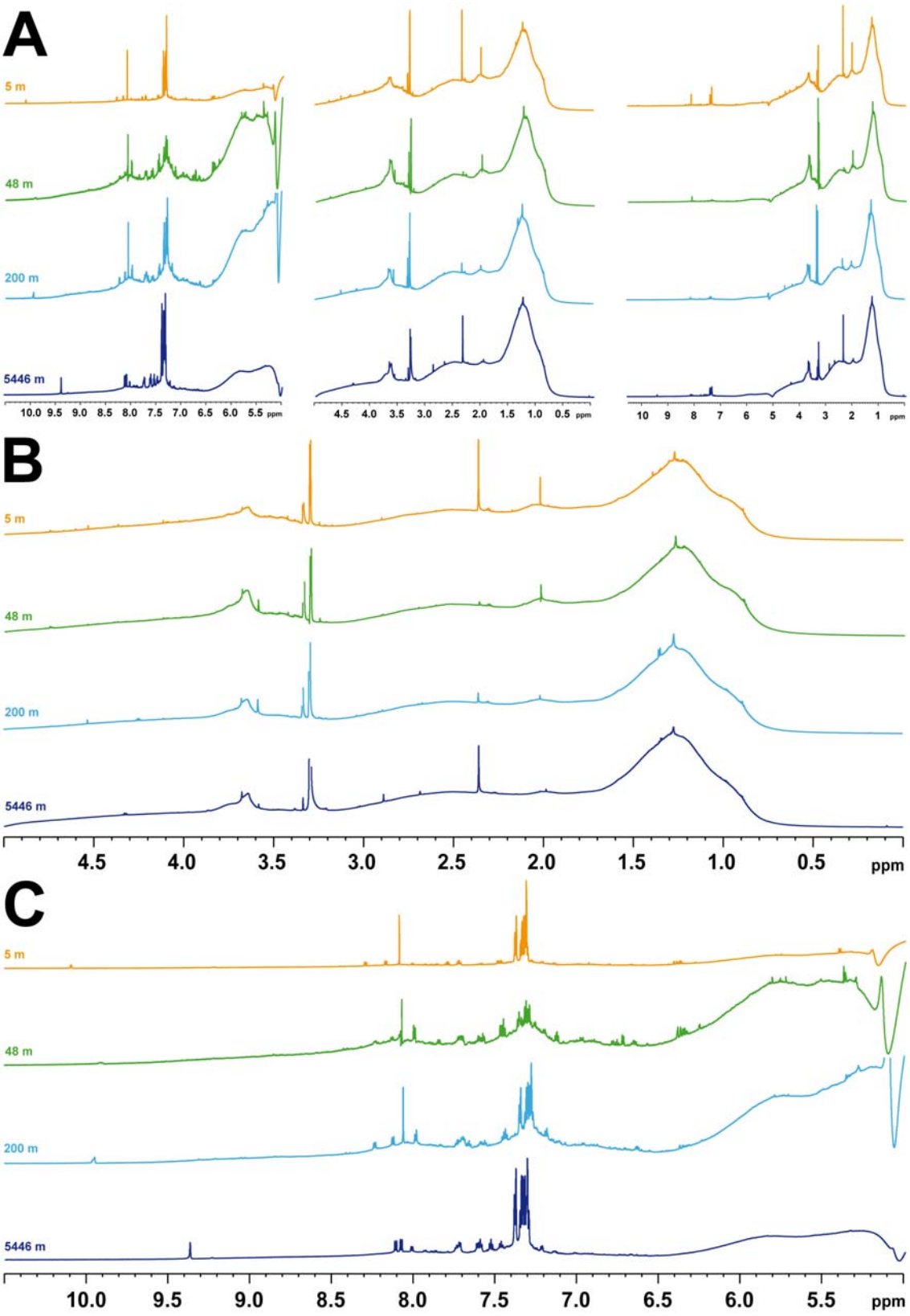
SUPPORTING ONLINE FIGURES AND TABLES

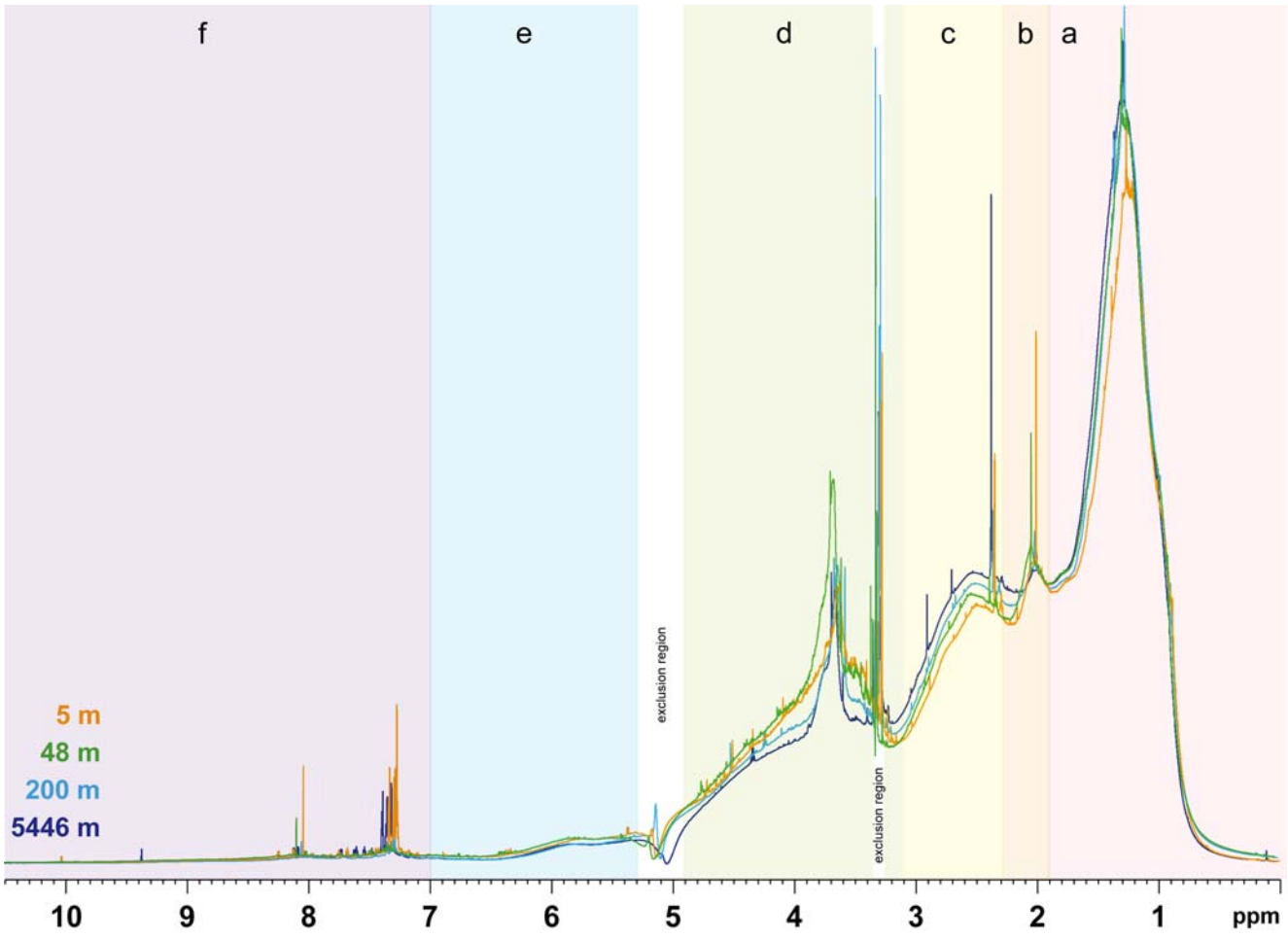
spectrum	Figure	PK	NS	AQ [ms]	D1 [ms]	NE	WDW1	WDW2	PR1	PR2
^{1}H,^{1}H TOCSY	1A, 1B	QCI	16	750	3250	1600	SI	EM	2.5	7.5
^{1}H,^{1}H TOCSY	1C, 1D	QCI	16	750	3250	1600	SI	GM	6	-0.4 / 0.6
^{1}H NMR	3	QCI	512	5000	10000	-	-	EM	-	1
^{13}C NMR	5 m	QCO	36064	1000	19000	-	-	EM	-	12.5 / 5 / 1
^{13}C NMR	48 m	QCO	22432	1000	19000	-	-	EM	-	12.5 / 5 / 1
^{13}C NMR	200 m	QCO	21146	1000	19000	-	-	EM	-	12.5 / 5 / 1
^{13}C NMR	5446 m	QCO	10640	1000	19000	-	-	EM	-	12.5 / 5 / 1
^{13}C DEPT	5A : FISH_A	QCI	16384	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5A : FISH_B	QCI	32768	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5A : DEPT-135	QCI	16384	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5B : FMAX_A	QCI	14400	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5B : FMAX_B	QCI	28800	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5B : DEPT-135	QCI	14400	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5C : 200_A	QCI	17920	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5C : 200_B	QCI	35840	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5C : DEPT-135	QCI	17920	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5D : 5446_A	QCI	25632	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5D : 5446_B	QCI	23937	1000	2000	-	-	EM	-	12.5
^{13}C DEPT	5D : DEPT-135	QCI	25345	1000	2000	-	-	EM	-	12.5
^{1}H,^{1}H JRES	6A, 6B	QCI	80	750	750	80	QS	QS	0	0
^{1}H,^{1}H JRES	6C, 6D	QCI	128	750	750	80	QS	QS	0	0
^{1}H,^{1}H JRES	6E, 6F	QCI	80	750	750	80	QS	QS	0	0
^{1}H,^{1}H JRES	6G, 6H	QCI	128	750	750	80	QS	QS	0	0
^{1}H,^{1}H COSY	7A, 7B	QCI	16	750	2250	1600	QS	EM	3	2
^{1}H,^{1}H COSY	7C, 7D	QCI	64	750	2250	1600	QS	EM	2.5	2.5
^{1}H,^{1}H COSY	7E, 7F	QCI	16	750	2250	1600	QS	EM	3	2
^{1}H,^{1}H COSY	7G, 7H	QCI	64	750	2250	1600	QS	EM	2.5	2.5
^{1}H,^{13}C DEPT-HSQC	CH_3 : 8A	QCI	64	250	1250	1024	QS	EM	2.5	2.5
^{1}H,^{13}C DEPT-HSQC	CH_3 : 8B	QCI	64	250	1250	1024	QS	EM	2.5	2.5
^{1}H,^{13}C DEPT-HSQC	CH_3 : 8C	QCI	64	250	1250	1024	QS	EM	2.5	2.5
^{1}H,^{13}C DEPT-HSQC	CH_3 : 8D	QCI	64	250	1250	1024	QS	EM	2.5	2.5
^{1}H,^{13}C DEPT-HSQC	CH_2 : 8E	QCI	64	250	1250	1024	QS	EM	2.5	2.5
^{1}H,^{13}C DEPT-HSQC	CH_2 : 8F	QCI	64	250	1250	1024	QS	EM	2.5	2.5
^{1}H,^{13}C DEPT-HSQC	CH_2 : 8G	QCI	64	250	1250	1024	QS	EM	2.5	2.5
^{1}H,^{13}C DEPT-HSQC	CH_2 : 8H	QCI	64	250	1250	1024	QS	EM	2.5	2.5

spectrum	Figure	PK	NS	AQ [ms]	D1 [ms]	NE	WDW1	WDW2	PR1	PR2
$^{1}H,^{13}C$ DEPT-HSQC	CH_3 : 9A,9C	QCI	64	250	1250	1024	QS	EM	2.5	2.5
$^{1}H,^{13}C$ DEPT-HSQC	CH_2 : 9B	QCI	64	250	1250	1024	QS	EM	2.5	2.5
$^{1}H,^{13}C$ HSQC-TOCSY	10A	QCI	288	250	1250	394	QS	EM	2	2
$^{1}H,^{13}C$ HSQC-TOCSY	10B, 10E	QCI	320	250	1250	400	QS	EM	2.5	2.5
$^{1}H,^{13}C$ HSQC-TOCSY	10C	QCI	64	250	1250	1024	QS	EM	2.5	2.5
$^{1}H,^{13}C$ HSQC-TOCSY	10D	QCI	320	250	1250	400	QS	EM	2.5	12.5
$^{1}H,^{13}C$ HMBC	11A, 11B	QCI	4000	250	1250	117	QS	EM	2.5	5
$^{1}H,^{13}C$ HMBC	11C, 11D	QCI	4000	750	750	161	QS	EM	2.5	5
$^{1}H,^{1}H$ TOCSY	13A, 13E	QCI	16	750	3250	1600	SI	EM	2.5	7.5
$^{1}H,^{1}H$ TOCSY	13B, 13F	QCI	32	750	3250	1600	SI	EM	2.5	7.5
$^{1}H,^{1}H$ TOCSY	13C, 13G	QCI	8	750	3250	1600	SI	EM	2.5	7.5
$^{1}H,^{1}H$ TOCSY	13D, 13H	QCI	32	750	3250	1600	SI	EM	2.5	7.5
$^{1}H,^{13}C$ HSQC	14A, 14E, 14I	QCI	400	250	1250	137	QS	EM	2.5	5
$^{1}H,^{13}C$ HSQC	14B, 14F	QCI	400	250	1250	142	QS	EM	2.5	5
$^{1}H,^{13}C$ HSQC	14C, 14G	QCI	800	250	1250	137	QS	EM	2.5	5
$^{1}H,^{13}C$ HSQC	14D, 14H	QCI	1800	250	1250	140	QS	EM	2.5	5

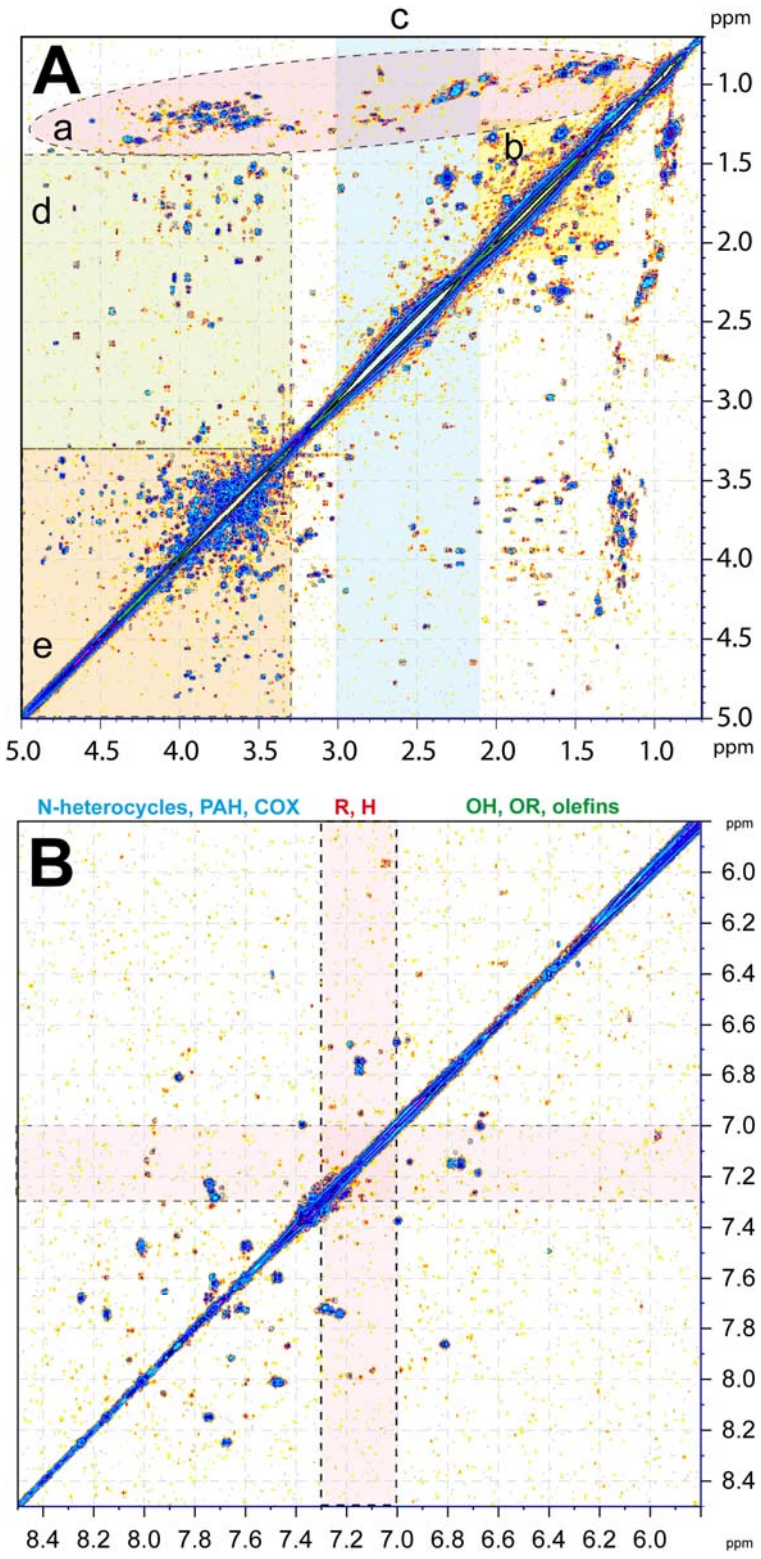
Supporting Online Table 1: Acquisition parameters of NMR spectra, shown according to figures. PK: probeheads used for acquisition of NMR spectra, QCI: cryogenic inverse geometry 5 mm z-gradient $^{1}H/^{13}C/^{15}N/^{31}P$ QCI probe (B_0 = 18.8 T); QCO: cryogenic classical geometry 3 mm z-gradient $^{1}H/^{13}C/^{15}N/^{31}P$ probe; TXI: cryogenic inverse geometry 5 mm z-gradient ^{1}H, ^{13}C, ^{15}N probe (B_0 = 11.7 T); NS: number of scans (for 2D NMR: F2); AQ: acquisition time [ms]; D1: relaxation delay [ms]; NE: number of F1 increments in 2D NMR spectra; WDW1, WDW2: apodization functions in F1/ F2 (EM/GM: line broadening factor [Hz]; QS: shifted square sine bell; SI: sine bell); PR1, PR2: coefficients used for windowing functions WDW1, WDW2, EM/GM are given in [Hz], SI/QS derived functions indicate shift by π/n.



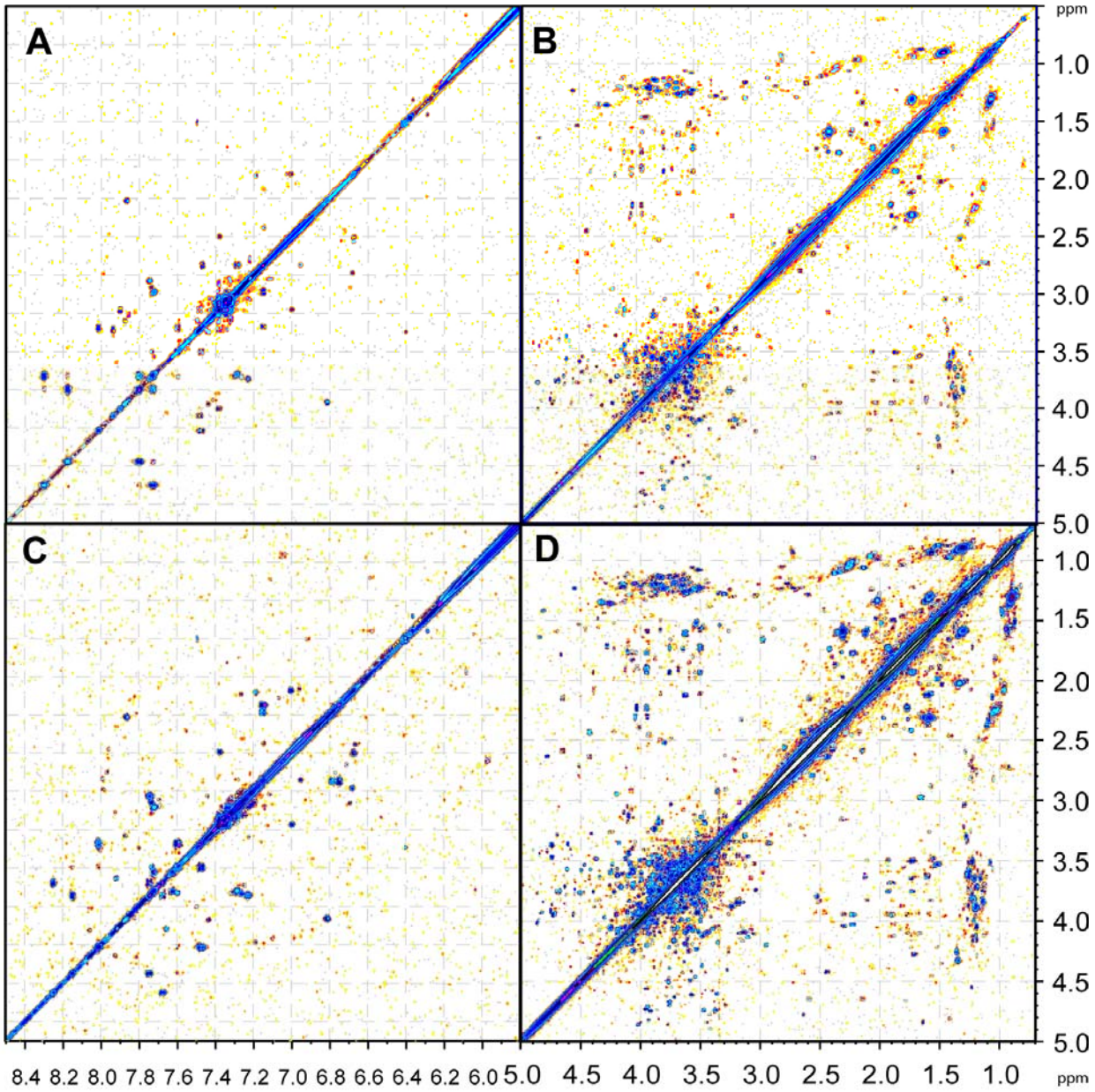
Supporting Online Fig. 1. ^{1}H NMR spectra of marine DOM obtained by solid phase extraction (PPL) from 3.1°E; -17.7°S (Angola basin) at different depths according to color: orange: 5 m (FISH, near surface photic zone); green: 48 m (FMAX, fluorescence maximum); blue: 200 m (upper mesopelagic zone); dark blue: 5446 m (30 m above ground) with respective spectral intensities scaled to 100% of maximum intensity (with methanol excluded) within the regions shown. (A): three spectral ranges, δ_H = 5…10.5 / 0…5 / 0…10.5 ppm; (B): entire spectral range, δ_H = 0…10.5 ppm. (C): unsaturated protons, bound to sp^2-hybridized carbon; δ_H = 5…10.5 ppm and anomeric protons (δ_H < 5.5 ppm); cf. Fig. 3.



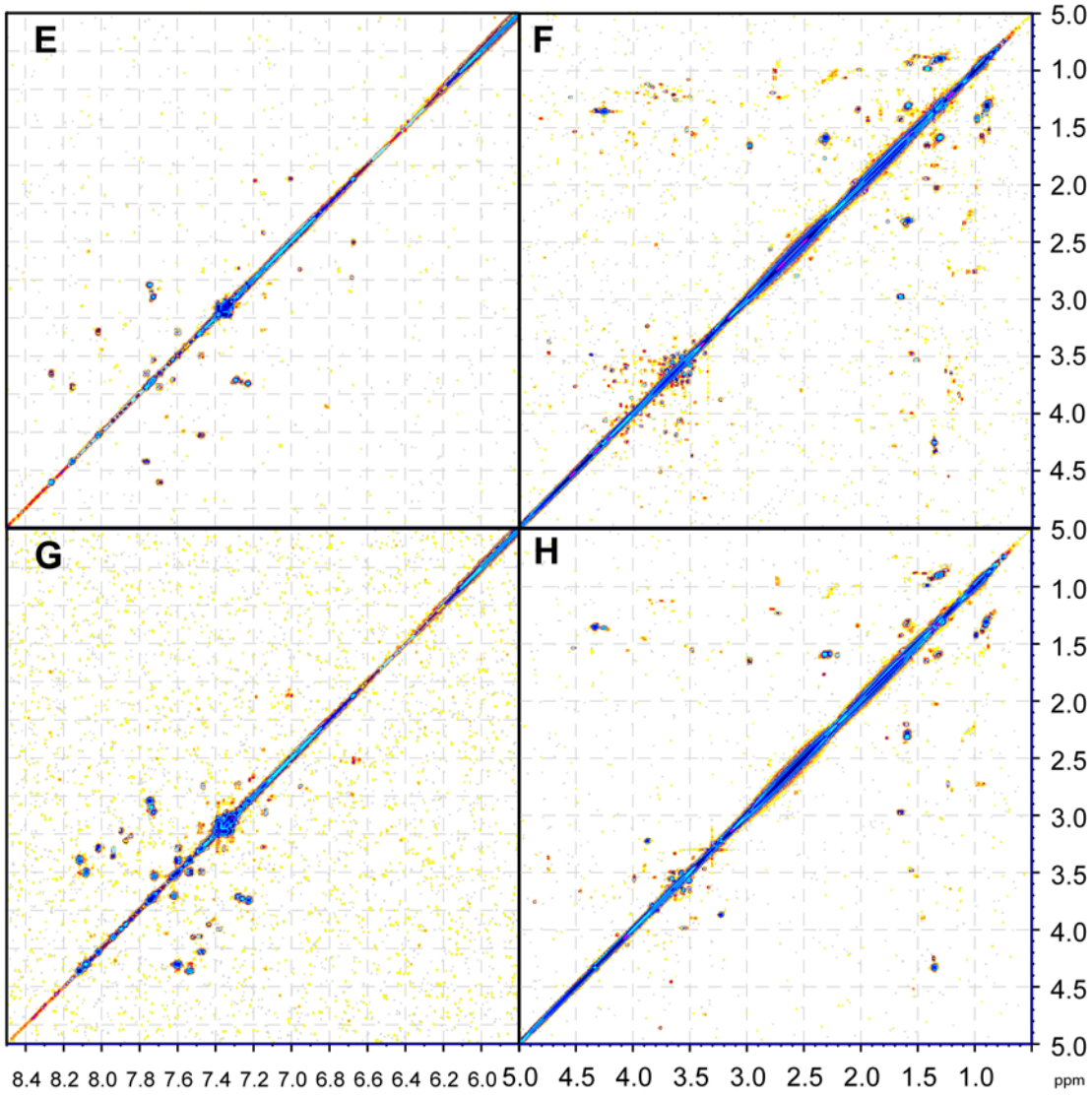
Supporting Online Fig. 2. ^{1}H NMR spectra of four marine DOM acquired with solvent suppression and exclusion regions used in the computation of NMR section integrals and overlay NMR spectra (Fig. 3 and this figure) which denote HD_2COD and residual HDO. Intensities are normalized to 100% total integral in the entire chemical shift range shown (δ_H = 0…10.5 ppm). Fundamental substructures are indicated from higher to lower field (from right to left), (a) aliphatics, **H**CCC; (b) "acetate-analogue", $\underline{\mathbf{H}}_3$CC(=O)-O-; (c) carboxyl-rich alicyclic materials (CRAM), **H**C(C)-COX; (d) "carbohydrate-like" and methoxy, **H**CO; (e) olefinic, **H**C=C; and (f) aromatic NMR resonances **H**C_{ar}. Superimposed small NMR resonances indicative of comparatively abundant biological and biogeochemical molecules were most significant in the aromatic section (f), well noticeable in sections (d) and (e) and of continual lesser occurrence in the order c > b > a.



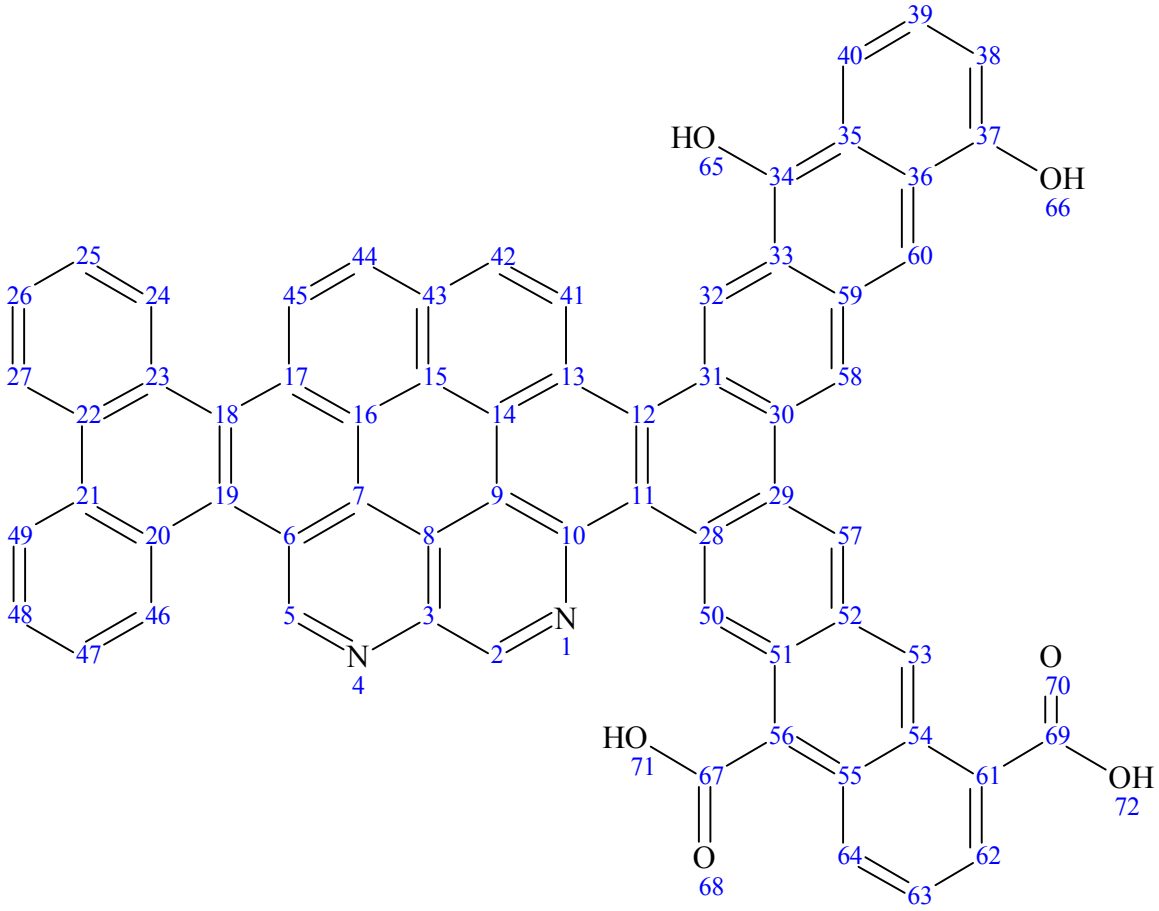
Supporting Online Fig. 3. ^{1}H, ^{1}H COSY NMR spectrum of marine surface DOM FMAX (cf. Fig. 7C, 7D) with key substructures indicated. Panel A: aliphatic section; section a: **H**$_3$CC**H** cross peaks related to chain terminating methyl groups, for δ_H > 3 ppm with oxygenated carbon (**H**$_3$CC**H**-O-); section b: intra aliphatic HC-**H**C-**H**C-CH cross peaks excluding methyl; section c: **H**CC**H**-COX cross peaks with carbonyl derivatives; section d: functionalized aliphatics connected with oxygenated carbon Z-C-**H**CC**H**-O [Z = O (N)]; section e: -O-**H**CC**H**-O- cross peaks, carbohydrates and esters, ethers and alcohols. Panel B: aromatic section with key substituents provided: electron withdrawing, polycyclic aromatic hydrocarbons and six-membered N-heterocycles (δ_H > 7.3 ppm), neutral (δ_H ~ 7…7.3 ppm), electron donating, olefins and five membered heterocycles (δ_H < 7 ppm); aromatic COSY cross peaks represent rather abundant molecular signatures of marine DOM.



Supporting Online Fig. 4A. $^{1}H,^{1}H$ COSY NMR spectra of (A, C) downfield (δ_H = 5.8...8.5 ppm; aromatic and olefinic C-C_{sp2}**H**-C_{sp2}**H**-C cross peaks) and (B, D) upfield ^{1}H NMR chemical shift region (δ_H = 0.5…5.0 ppm; aliphatic C-**H**C-**H**C-X (X: C, N, O); A, B: 5 m (FISH, near surface photic zone); C, D: 48 m (FMAX, fluorescence maximum).



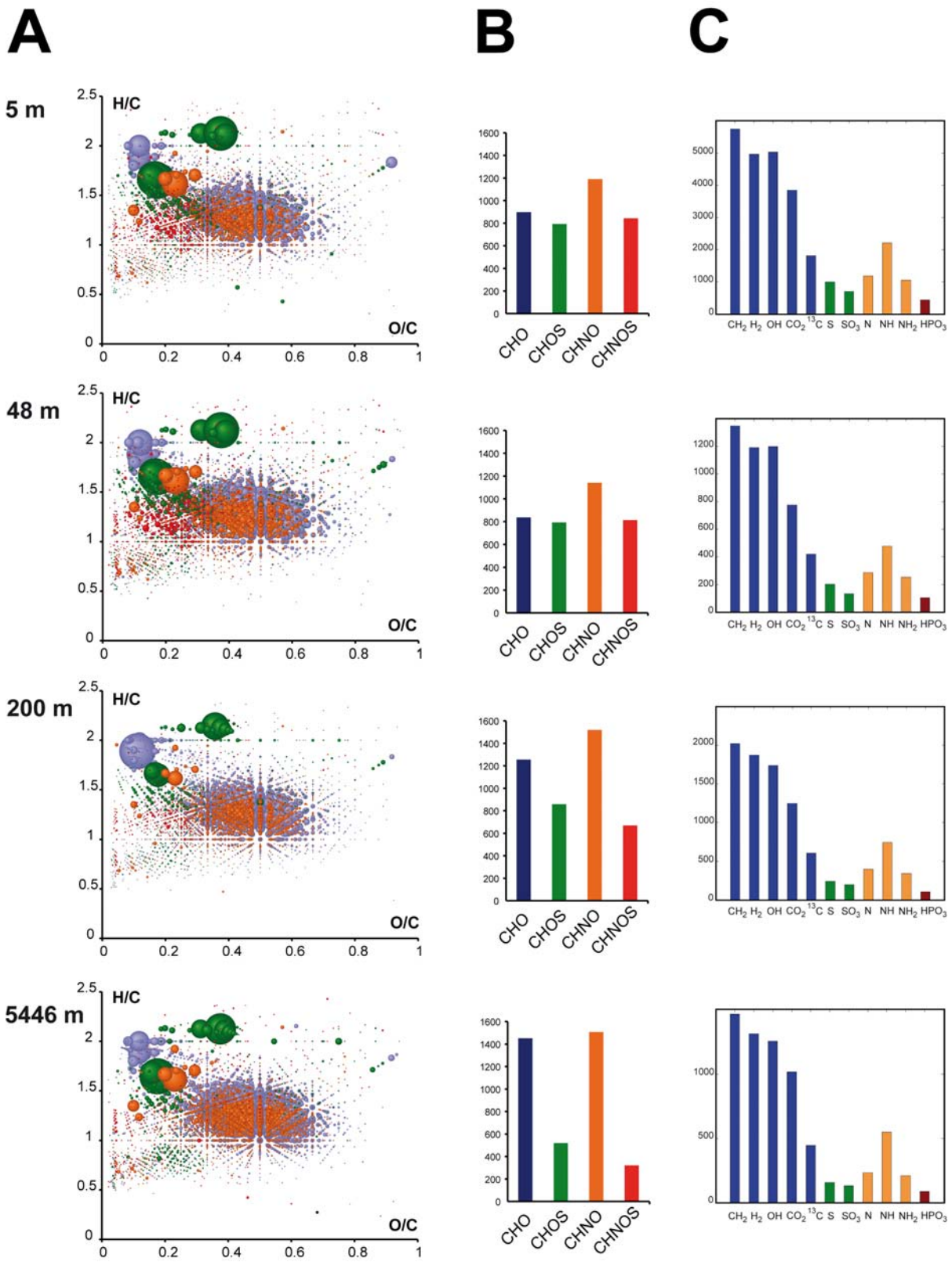
Supporting Online Fig. 4B. ^{1}H,^{1}H COSY NMR spectra of (E, G) downfield (δ_H = 5.8...8.5 ppm; aromatic and olefinic C-C_{sp2}**H**-C_{sp2}**H**-C cross peaks) and (F, H) upfield ^{1}H NMR chemical shift region (δ_H = 0.5…5.0 ppm; aliphatic C-**H**C-**H**C-X (X: C, N, O); E, F: 200 m (upper mesopelagic zone); G, H: 5446 m (30 m above ground).



Supporting Online Fig. 5. ACD software proposed numbering scheme of PAH model molecule $C_{65}H_{34}N_2O_6$ (IUPAC mass. 938.975 Da; cf. Fig. 12 and Supporting Online Table 2).

carbon number	CH_n	δ(^{13}C) [ppm]	δ(^{1}H) [ppm]	carbon number	CH_n	δ(^{13}C) [ppm]	δ(^{1}H) [ppm]	carbon number	CH_n	δ(^{13}C) [ppm]	δ(^{1}H) [ppm]
2	CH	164.32	9.18	25	CH	126.55	7.75	47	CH	129.36	7.72
3	C	151.49		26	CH	126.44	7.61	48	CH	126.44	7.61
5	CH	154.56	9.90	27	CH	125.15	8.57	49	CH	126.78	8.66
6	C	130.92		28	C	140.71		50	CH	136.73	9.43
7	C	131.05		29	C	136.60		51	C	141.62	
8	C	132.93		30	C	147.56		52	C	137.65	
9	C	133.41		31	C	130.89		53	CH	121.83	8.61
10	C	141.35		32	CH	136.41	8.86	54	C	134.91	
11	C	133.85		33	C	127.98		55	C	134.91	
12	C	132.19		34	C	150.72		56	C	140.72	
13	C	135.02		35	C	128.97		57	CH	134.28	9.52
14	C	134.79		36	C	128.97		58	CH	125.79	8.83
15	C	148.67		37	C	154.47		59	C	139.28	
16	C	132.73		38	CH	109.77	7.04	60	CH	110.67	8.84
17	C	127.11		39	CH	123.76	7.14	61	C	134.90	
18	C	123.78		40	CH	123.22	7.94	62	CH	132.45	8.7
19	C	127.60		41	CH	131.74	9.26	63	CH	129.81	7.47
20	C	131.30		42	CH	132.58	8.47	64	CH	133.78	8.47
21	C	141.33		43	C	131.74		67	C	169.89	
22	C	131.79		44	CH	132.58	8.47	69	C	168.82	
23	C	138.69		45	CH	129.05	9.13				
24	CH	132.52	9.07	46	CH	132.12	9.14				

Supporting Online Table 2: computed proton and carbon NMR chemical shift of PAH model $C_{65}H_{34}N_2O_6$ (IUPAC mass. 938.975 Da; cf. Fig. 12 and Supporting Online Fig. 5)



Supporting Online Fig. 6. Negative electrospray 12T FTICR mass spectra of marine DOM samples; from top to bottom: 5 m (FISH, near surface photic zone); 48 m (FMAX, fluorescence maximum); 200 m (upper mesopelagic zone); 5446 m (30 m above ground). Panel A: H/C versus O/C van Krevelen diagrams of FTICR mass spectra from 150-700 Da with color code as provided in panel B: histograms represent counts of respective unique CHO, CHOS, CHNO and CHNOS containing molecules (cf. text). Panel C: histograms denoting frequency of major fragments in FTICR mass spectra (Schmitt-Kopplin et al., 2010a).

metal	FISH	FMAX	200 m	5446 m
Co	59	48	309	72
Cr	1626	1016	1760	1676
Cu	1407	4927	1359	2187
Fe	75305	7375	3057	7327
Mn	3080	126	1230	255
Ni	10339	7130	765	586
Al	29937	9884	13250	19731
As	12191	13678	13525	5740
B	42282	22644	7038	94615
Ba	870	1310	1637	4500
Ca	49380	44064	47124	136153
Cd	237	135	106	779
Hg	61	47	21	23
Mg	19042	11108	36414	42404
Mo	15092	8629	7252	8711
Na	351834	169831	172586	481727
P	270357	200432	138007	79903
Pb	1117	842	1178	172
S	1145004	813967	645666	435574
Sb	171	104	133	100
Sn	151	164	228	59
Sr	302	373	334	395
Th	45	21	20	12
Ti	512	334	1655	199
U	1673	805	456	372
Zn	10432	14443	10159	20365
Be	4	4	4	3
Bi	4	3	3	3
Cs	4	4	4	3
K	33332	32742	32436	28846
Li	722	710	707	626
Se	4290	4192	4162	3692
V	1710	1677	1671	1480

Supporting Online Table 3. Metal concentration (ng metal / L methanol extract) for four marine DOM samples (gray cells: metal concentrations were below detection limit; cf. Fig. 17).