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Consistent increase in dimethyl sulfide (DMS) in response to high CO_2 in five shipboard bioassays from contrasting NW European waters

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Consistent increase in dimethyl sulphide (DMS) in response to high CO_2 in five shipboard bioassays from contrasting NW European waters.

Supplementary Material

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Supplementary Tables and Figures

Table S1. Comparison of carbonate chemistry parameters determined from bioassay 0 h measurements, and the subsequent CTD cast performed at the same station and depth, 2 – 3 h after the primary bioassay cast. n.d.= no data available.

	DIC (µmol kg^{-1})		TA (µmol kg^{-1})		*p*H (total)		pCO_2 (µatm)	
Expt. I.D.	Bioassay 0 h (n = 1)	CTD 0 h Mean(SD) (n = 3)	Bioassay 0 h (n = 1)	CTD 0 h Mean(SD) (n = 3)	Bioassay 0 h (n = 1)	CTD 0 h Mean(SD) (n = 3)	Bioassay 0 h (n = 1)	CTD 0 h Mean(SD) (n = 3)
E01	2091.9	2091.8 (0.9)	2310.9	2310.9 (2.3)	8.11	8.11 (0.01)	342.3	342.6 (4.2)
E02	n.d.	2094.6 (1.0)	n.d.	2322.2 (2.4)	n.d.	8.12 (0.01)	n.d.	333.7 (5.4)
E03	2084.1	2083.8 (0.6)	2343.5	2347.1 (3.6)	8.11	8.11 (0.01)	345.4	339.8 (6.0)
E04	2085.7	2085.5 (1.6)	2298.7	2295.6 (0.4)	8.05	8.05 (0.01)	395.4	400.6 (5.7)
E05	2084.9	2084.6 (1.5)	2307.9	2311.5 (3.6)	8.07	8.08 (0.01)	374.7	368.1 (4.4)

Table S2. Summary of results from one-way analyses of variance (ANOVA) on DMS and DMSPt concentrations under four CO_2 treatments (ambient, ~550 µatm, ~750 µatm, ~1000 µatm) from five bioassay experiments at each sampling time point (48 h and 96 h). Values shown include the *F* ratio of mean squares from the ANOVA, and *T* values from Holm-Sidak pairwise comparisons between all treatments. *df* = 12, superscript letters indicate level of significance: [a] = $P<0.05$, [b] = $P<0.01$, [c] = $P<0.001$.

	F ratio		*T* value					
[DMS]			550		750		1000	
	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h
E01	33.50[c]	3.92						
Ambient			4.16[a]	n.s.	5.50[b]	n.s.	7.69[c]	n.s.
550			-	-	n.s.	n.s.	6.53[a]	n.s.
750			-	-	-	-	n.s.	n.s.
E02	26.50[c]	33.50[c]						
Ambient			3.68[a]	3.87[b]	5.40[b]	9.68[c]	8.75[c]	6.45[c]
550			-	-	n.s.	5.81[b]	5.07[b]	2.59[a]
750			-	-	-	-	3.34[a]	3.23[a]
E03	27.13[c]	11.20[b]						
Ambient			3.60[b]	n.s.	5.74[b]	n.s.	8.76[c]	n.s.
550			-	-	n.s.	n.s.	5.46[b]	5.24[b]
750			-	-	-	-	3.02[b]	4.63[b]
E04	4.91[a]	11.46[b]						
Ambient			n.s.	5.57[b]	n.s.	4.17[b]	3.60*	n.s.
550			-	-	n.s.	n.s.	n.s.	3.64[b]
750			-	-	-	-	n.s.	n.s.
E05	7.14[b]	n.s.						
Ambient			n.s.	n.s.	n.s.	n.s.	4.51[b]	n.s.
550			-	-	n.s.	n.s.	n.s.	n.s.
750			-	-	-	-	n.s.	n.s.
[DMSPt]								
E01	5.80[a]	n.s.						
Ambient			n.s.	n.s.	n.s.	n.s.	3.66[a]	n.s.
550			-	-	n.s.	n.s.	n.s.	n.s.
750			-	-	-	-	n.s.	n.s.
E02	n.s.	17.84[c]						
Ambient			n.s.	4.29[a]	n.s.	5.61[b]	n.s.	6.87[c]
550			-	-	n.s.	n.s.	n.s.	n.s.
750			-	-	-	-	n.s.	n.s.
E03	15.93[c]	16.39[c]						
Ambient			4.48[b]	n.s.	5.71[b]	4.49[b]	6.18[b]	6.72[c]
550			-	-	n.s.	n.s.	n.s.	4.24[a]
750			-	-	-	-	n.s.	n.s.
E04	118.17[c]	n.s.						
Ambient			9.51[c]	n.s.	14.57[c]	n.s.	17.09[c]	n.s.
550			-	-	5.16[b]	n.s.	8.58[c]	n.s.
750			-	-	-	-	3.97[b]	n.s.
E05	n.s.	102.18[c]						
Ambient			n.s.	4.62[b]	n.s.	12.71[c]	n.s.	15.47[c]
550			-	-	n.s.	8.10[c]	n.s.	10.85[c]
750			-	-	-	-	n.s.	2.75[a]

Table S3. Significance of relationships between DMS(P) parameters and $[H^+]$ (x 10^{-8} equivalents L^{-1}) for each experimental bioassay, and for all experimental data combined. Values shown are the coefficient of determination (r^2) and the significance of the *F*-ratio of the *ANOVA* of the regression ($^a = P < 0.05$, $^b = P < 0.01$, $^c = P < 0.001$, n.s. = not significant). For single experiments, $n = 12$. For combined data set $n = 60$. The majority of relationships were linear but those marked * were best described with a polynomial fit.

	Time (h)	E01	E02	E03	E04	E05	All data
DMS	48	87.2^c	90.9^c	91.5^c	58.4^b	65.0^b	37.8^{c*}
	96	34.9^a	52.6^b	79.3^{b*}	n.s.	49.6^a	n.s.
DMSPt	48	43.0^a	n.s.	-74.6^c	87.3^c	-33.9^a	-28.3^c
	96	n.s.	-84.3^c	-86.8^c	n.s.	-93.5^c	-25.7^{c*}
DMS: DMSPt	48	83.3^c	57.2^b	92.7^c	85.3^c	81.1^c	57.4^{c*}
	96	n.s.	77.3^c	89.9^{c*}	n.s.	92.6^{c*}	68.1^{c*}
DMS: Chl *a*	48	93.4^c	87.9^c	86.3^c	42.5^a	74.3^c	37.8^{c*}
	96	-39.3^a	63.8^b	78.4^{b*}	-81.8^{c*}	92.8^c	59.4^{c*}
DMSPt: Chl *a*	48	-52.0^b	n.s.	n.s.	-62.0^b	n.s.	n.s.
	96	-56.1^b	-63.4^b	-62.7^b	-64.6^b	n.s.	n.s.

Table S4. Summary of results from one-way analyses of variance (ANOVA) on small phytoplankton abundances (<10 μm, pico- and nano-phytoplankton) and total bacteria abundances under four CO_2 treatments from five bioassay experiments at each sampling time point (48 h and 96 h). Values shown include *F* ratio of mean squares from ANOVA, and *T* values from Holm-Sidak pairwise comparisons between ambient and high CO_2 treatments. *df* = 12, superscript letters indicate level of significance: $^a = P<0.05$, $^b = P<0.01$, $^c = P<0.001$.

	F ratio		*T* value (significance of difference from ambient CO_2)					
			550		750		1000	
	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h
Small phytoplankton								
E01	n.s.	38.92^c	n.s.		n.s.	6.45^c	n.s.	9.33^c
E02	43.88^c	15.89^c	8.37^c	4.33^a	9.30^c	4.19^a	10.11^c	6.79^c
E03	5.64^a	16.13^c	4.09^a	5.15^b	n.s.	n.s.	n.s.	5.84^b
E04	69.33^c	9.46^{a*}	8.06^c	n.s.	11.78^c	n.s.	13.54^c	4.32^a
E05	60.52^c	6.04^a	12.07^c	3.46^a	11.23^c	n.s.	7.73^c	3.78^a
Total bacteria								
E01	n.s.	17.99^c	n.s.	n.s.	n.s.	5.43^b	n.s.	4.81^b
E02	55.50^c	28.65^c	6.96^c	5.62^b	12.88^c	7.67^c	6.99^c	8.34^c
E03	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
E04	n.s.	8.74^{a*}	n.s.	n.s.	n.s.	n.s.	n.s.	8.74^a
E05	2.26^a	39.38^c	n.s.	n.s.	n.s.	4.61^b	3.84^a	9.93^c

* = non-normal data, Kruskal Wallis ANOVA on ranks used

Table S5. Results of ANCOVA (test for homogeneity of regressions) performed on *de novo* DMSP synthesis rate constants (μDMSP, d^{-1}) to identify significant differences between ambient CO_2 and high CO_2 treatments. Data shown are *p* values of *F* ratio of ANCOVA. Significant differences where $p < 0.05$, and are indicated with an asterisk (*).

	Time (h)	550 µatm	750 µatm	1000 µatm
E01	48	*no data*	*no data*	*no data*
	96	*no data*	*no data*	*no data*
E02	48	7.70E-05*	4.40E-06*	8.60E-06*
	96	0.006*	1.40E-06**	1.60E-05*
E03	48	1.50E-05*	0.21	0.0014*
	96	0.85	0.19	0.121
E04	48	0.086	8.20E-05*	0.0097*
	96	2.00E-05*	0.00036*	0.385
E05	48	0.00034*	1.60E-09*	8.80E-11*
	96	0.16	0.62	4.40E-08*

Table S6. Summary of mean (SE) biological DMS consumption (BC), turnover time of DMS due to BC (τ_{BC}) and gross DMS production (GP) rates from triplicate dark incubations. Rates were determined at each sampling time point of each bioassay experiment (except 0h for E01). pCO_2 treatments: Ambient 335 – 395 µatm, high CO_2 (675 – 730 µatm). * = significantly different from ambient (*F* ratio from ANOVA $P < 0.05$).

		BC (nmol L^{-1} d^{-1})		τ_{BC} (d)		GP (nmol L^{-1} d^{-1})	
	time (h)	Ambient	~750µatm	Ambient	~750µatm	Ambient	~750µatm
E01	0	*no data*		*no data*		*no data*	
	48	2.8 (0.3)	7.3 (1.3) *	0.6 (0.1)	0.6 (0.2)	5.9 (0.8)	5.6 (0.8)
	96	1.9 (0.4)	2.3 (0.6)	5.0 (0.8)	3.0 (1.9)	4.1 (1.9)	2.1 (1.3)
E02	0	0.3 (0.1)		2.7 (0.7)		0.7 (0.1)	
	48	0.2 (0.1)	0.4 (0.1) *	11.8 (6.4)	8.9 (2.1)	-0.3 (0.1)	0.4 (0.2)
	96	0.6 (0.1)	0.9 (0.5)	4.4 (0.7)	12.3 (7.1)	0.4 (0.6)	3.4 (0.8) *
E03	0	1.2 (0.4)		2.3 (0.9)		4.9 (0.7)	
	48	1.9 (0.5)	1.3 (0.2)	1.4 (0.1)	5.0 (0.7) *	1.5 (0.1)	3.5 (0.4) *
	96	2.2 (0.5)	1.4 (0.6)	3.8 (0.7)	8.6 (5.8)	5.3 (2.0)	1.8 (0.4)
E04	0	0.6 (0.2)		2.0 (0.4)		0.1 (0.1)	
	48	0.4 (0.1)	0.5 (0.2)	4.0 (0.9)	5.2 (1.9)	2.3 (1.1)	2.7 (0.5)
	96	2.0 (0.2)	0.5 (0.1) *	2.5 (0.1)	6.2 (1.3) *	6.6 (1.0)	2.7 (0.3) *
E05	0	8.6 (1.6)		0.2 (0.04)		8.7 (1.4)	
	48	0.6 (0.3)	0.8 (0.3)	5.9 (2.0)	7.3 (2.0)	2.3 (1.1)	1.8 (0.1)
	96	1.8 (0.8)	0.9 (0.4) *	2.8 (1.1)	9.9 (3.6)	3.0 (0.9)	1.5 (0.9)

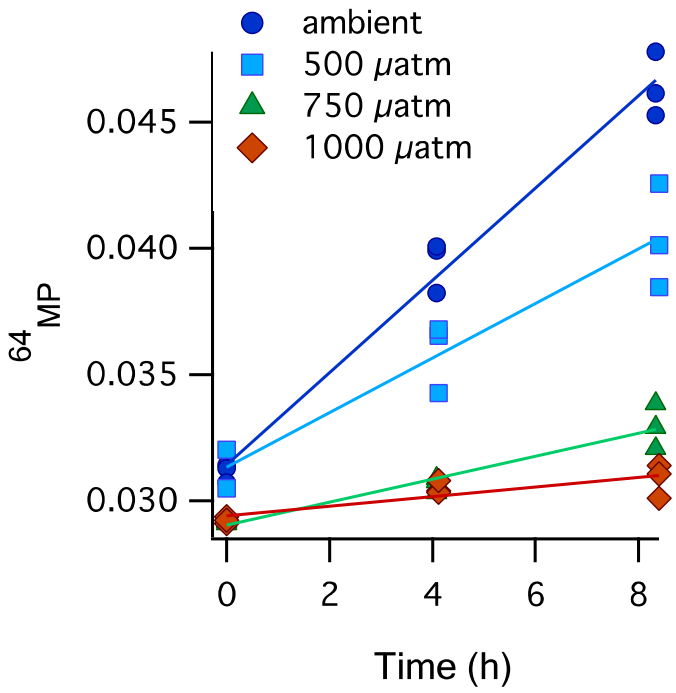

Figure S1. An example of the change in proportion of mass ratio 64 (^{64}MP) during incubations of water from experiment E05 at 48 h for the different pCO_2 treatments. Each point represents the data from a separate incubation.

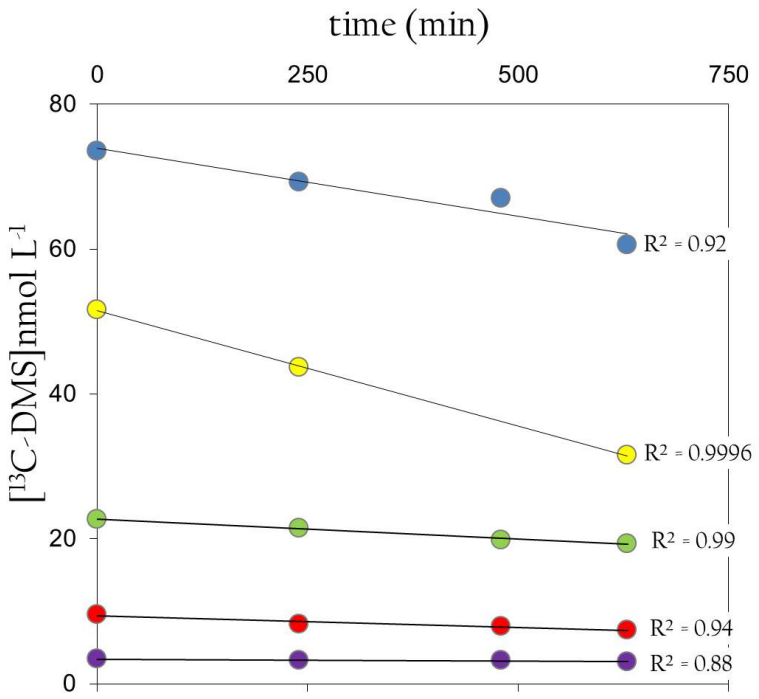

Figure S2. Linear change in ^{13}C-DMS concentrations (nmol L^{-1}) over the course of a 10.5 h concentration series incubation to determine the consumption kinetics. Data is for KE1.

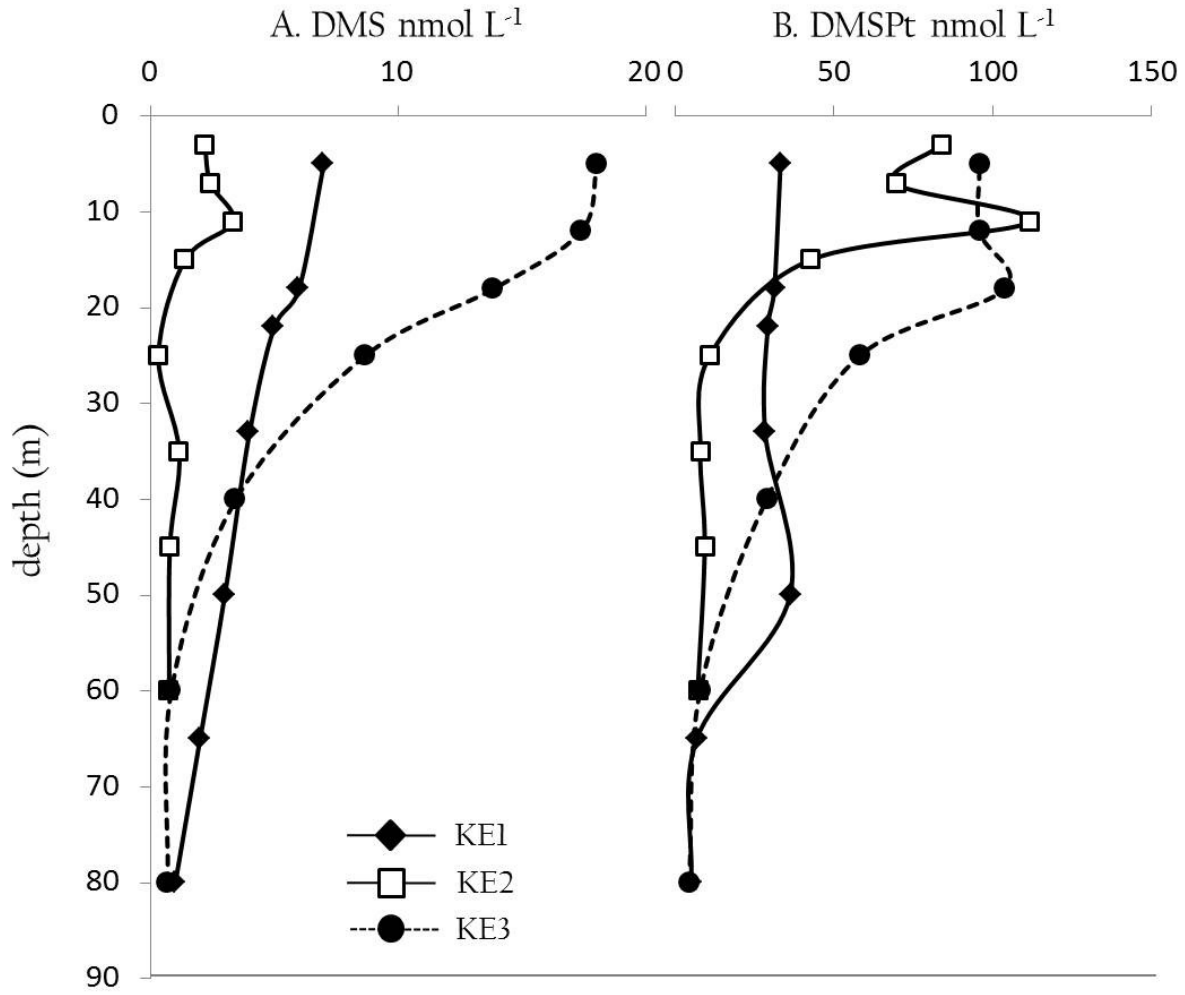

Figure S3. Depth profiles of DMS (nmol L^{-1}) and total DMSP (nmol L^{-1}) for casts relating to ^{13}C-DMS kinetic experiments. Water used in kinetic experiments was taken from surface Niskins (3 -5 m).

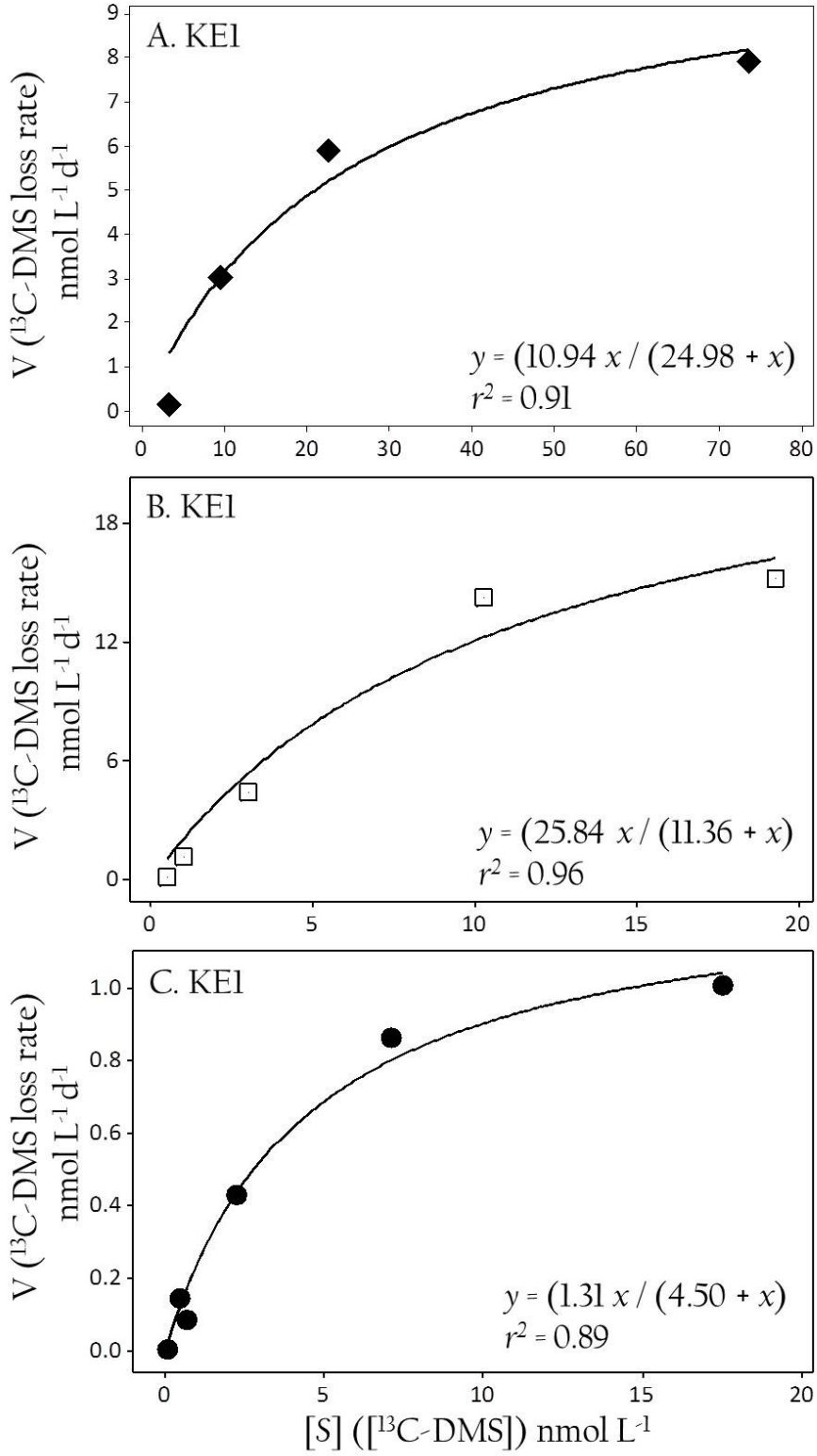

Figure S4. Kinetic curve for ^{13}C-DMS loss rates from dark incubations of whole seawater collected for three kinetic experiments. The data is fitted to the Michaelis-Menten equation derived through non-linear regression analysis. See Table S2 for details.

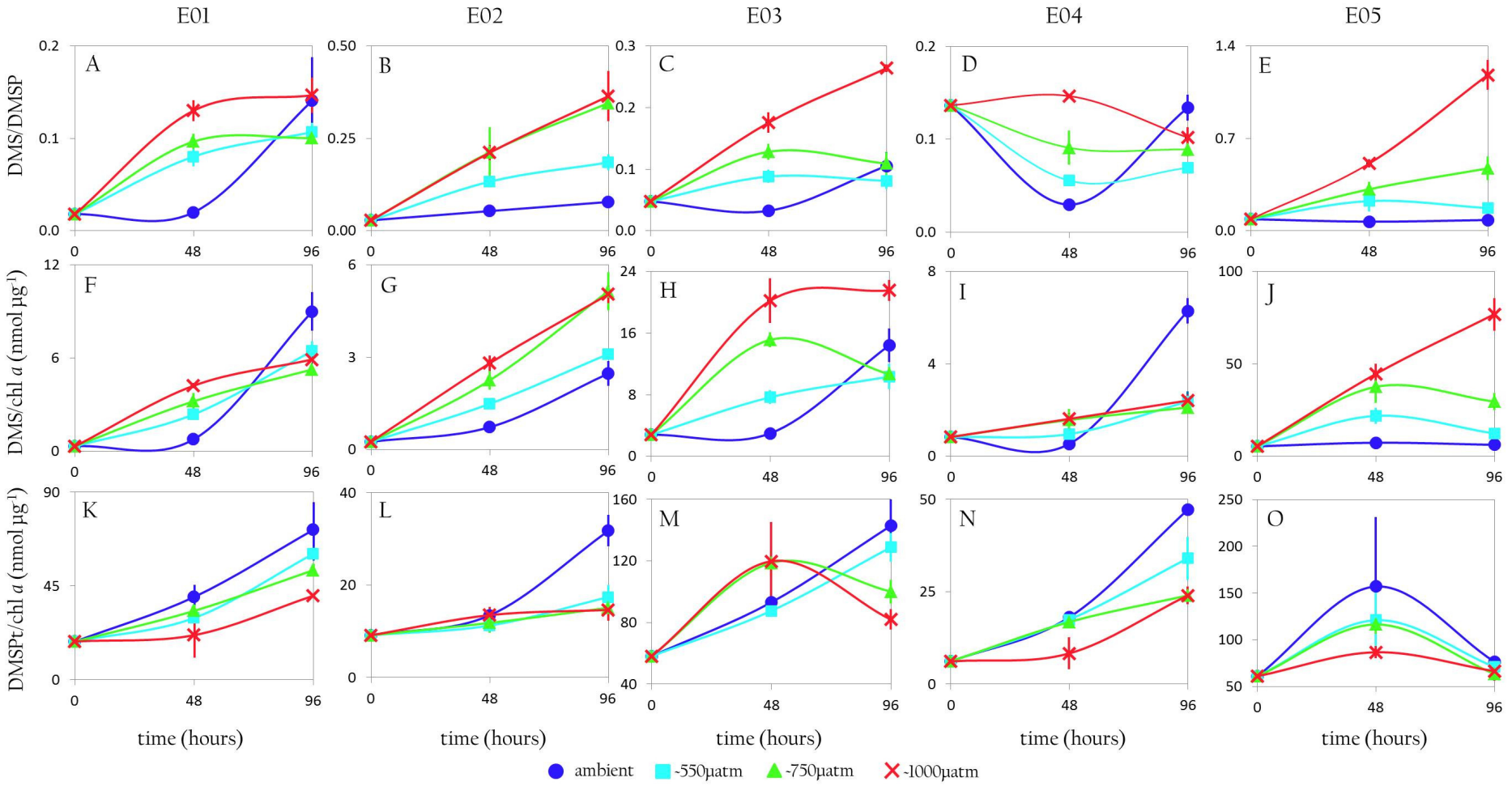

Figure S5. Ratios of DMS to DMSPt (A – E), DMS to Chl *a* (nmol µg^{-1}) (F – J) and DMSPt to Chl *a* (nmol µg^{-1}) (K – O) during five bioassay experiments. Values shown are means of experimental triplicates, and error bars indicate the standard error.