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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Biological DMS consumption: kinetic parameters

Three additional experiments, hereafter referred to as KE1, KE2 and KE3, were conducted to determine BC kinetic parameters. The locations of sampling for kinetic experiments are shown in Figure 1. Unfiltered surface seawater (3 – 5 m) was siphoned into five 1 L Tedlar bags that had been acid-washed with 1 % HCl and thoroughly rinsed three times with ultra-pure water. Once filling was complete, any bubbles and headspace were gently expelled from the bag. For each experiment, increasing concentrations of ^{13}C-DMS were added to each bag, ranging from at or below *in situ* concentrations up to ~74 nM. After the addition, the bags were left for one hour to allow complete homogenisation of the tracer. The bags were incubated for up to 12 h in the dark at *in situ* seawater temperature, and samples were processed and analysed as described above.

Initial DMS concentrations showed a wide range, from 1.0 nmol L^{-1} in KE1 to 16.8 nmol L^{-1} in KE3 (see Table S1 and water column profiles in Fig. S1). A large range of DMS: DMSPt ratios were also encountered, with a value of 0.2 for KE3 which was an order of magnitude higher than values of 0.02 and 0.03 for KE1 and KE2.

A summary of the Michaelis-Menten kinetic parameters for the three experiments, obtained through non-linear regression using Minitab 16.0 statistical software, are given in Table S1. Kinetic curves (see example Figure S2) were based on 5 to 6 data points; replicate samples were unfeasible due to the time taken to analyse all samples at each of four time points over a 12 h period. However, the non-linear regressions for each experiment were significant (P<0.05). The kinetic parameters generated in this study represent the activity of natural assemblages rather than the activity of single enzymes or species, and showed a similarly

broad range to *in situ* DMS(P) characteristics. Half saturation constants (K_s) ranged from 4.5 nmol L^{-1} in KE3 to 25.0 L^{-1} in KE1, with KE2 displaying an intermediate K_s value of 11.4 nmol L^{-1}. Thus, an inverse relationship between *in situ* DMS concentrations and K_s was apparent. Maximum DMS consumption rates (V_{max}) were also variable. KE3 displayed the lowest V_{max} of only 1.3 nmol L^{-1} d^{-1}, and this time KE2 gave the highest values with 25.9 nmol L^{-1} d^{-1}. Despite having the highest *Ks* and lowest *in situ* DMS concentrations, KE1 yielded an intermediate V_{max} of 10.9 nmol l^{-1} d^{-1}.

Supplementary Tables and Figures

Table S1. Kinetic parameters (K_s [half-saturation constant], V_{max} [maximum ^{13}C-DMS consumption rate] and turnover time of DMS due to BC (τ_{BC}) for ^{13}C-DMS loss rates at three contrasting sites in NW European shelf waters. Kinetic parameters were calculated by fitting data to the *Michaelis-Menten* equation through non-linear regression of loss rate vs. ^{13}C-DMS concentration data from three kinetic experiments (See Fig. S2). Initial *in situ* DMS concentrations measured at 0 h of each kinetic experiment are also shown.

Experiment	Date	Initial [DMS] (nmol L^{-1})	DMS:DMSPt	K_s ± SE (nmol L^{-1})	V_{max} ± SE (nmol L^{-1} d^{-1})	r^2	τ_{BC} (d)
KE1	19 June 11	1.0	0.02	25.0 ± 13.8	10.9 ± 2.5	0.91	0.09
KE2	24 June 11	3.8	0.03	11.4 ± 6.7	25.9 ± 7.3	0.96	0.15
KE3	05 July 11	16.8	0.2	4.5 ± 1.1	1.3 ± 0.1	0.89	12.9
			mean	13.6 ± 7.2	12.7 ± 3.3		

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. 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)

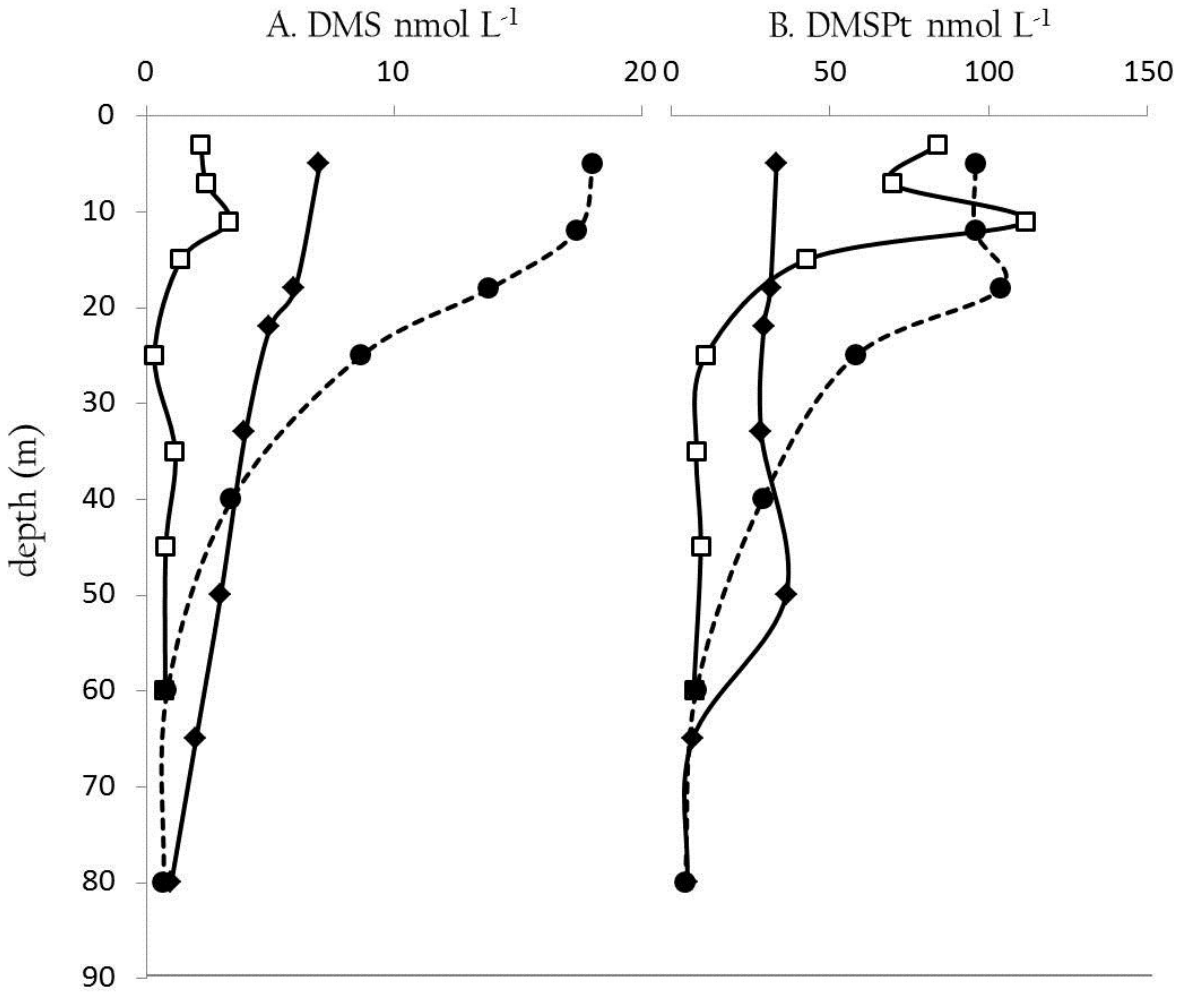

Figure S1. Depth profiles of DMS (nmol L^{-1}) and total DMSP (nmol L^{-1}) for casts relating to ^{13}C-DMS kinetic experiments. Water used in kinetic experiments was taken from surface Niskins (3 -5 m).

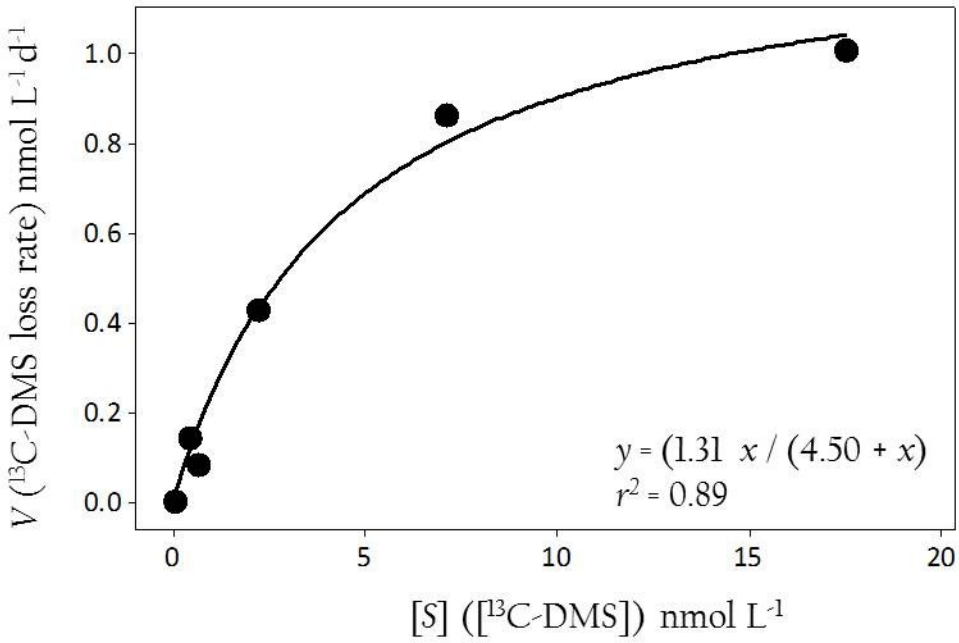

Figure S2. Kinetic curve for ^{13}C-DMS loss rates from dark incubations of whole seawater collected on 05 July 2011 (KE3). The data is fitted to the Michaelis-Menten equation derived through non-linear regression analysis. In this case, V_{max} was 1.31 nmol L^{-1} d^{-1}, and K_s was 4.50 nmol.

v

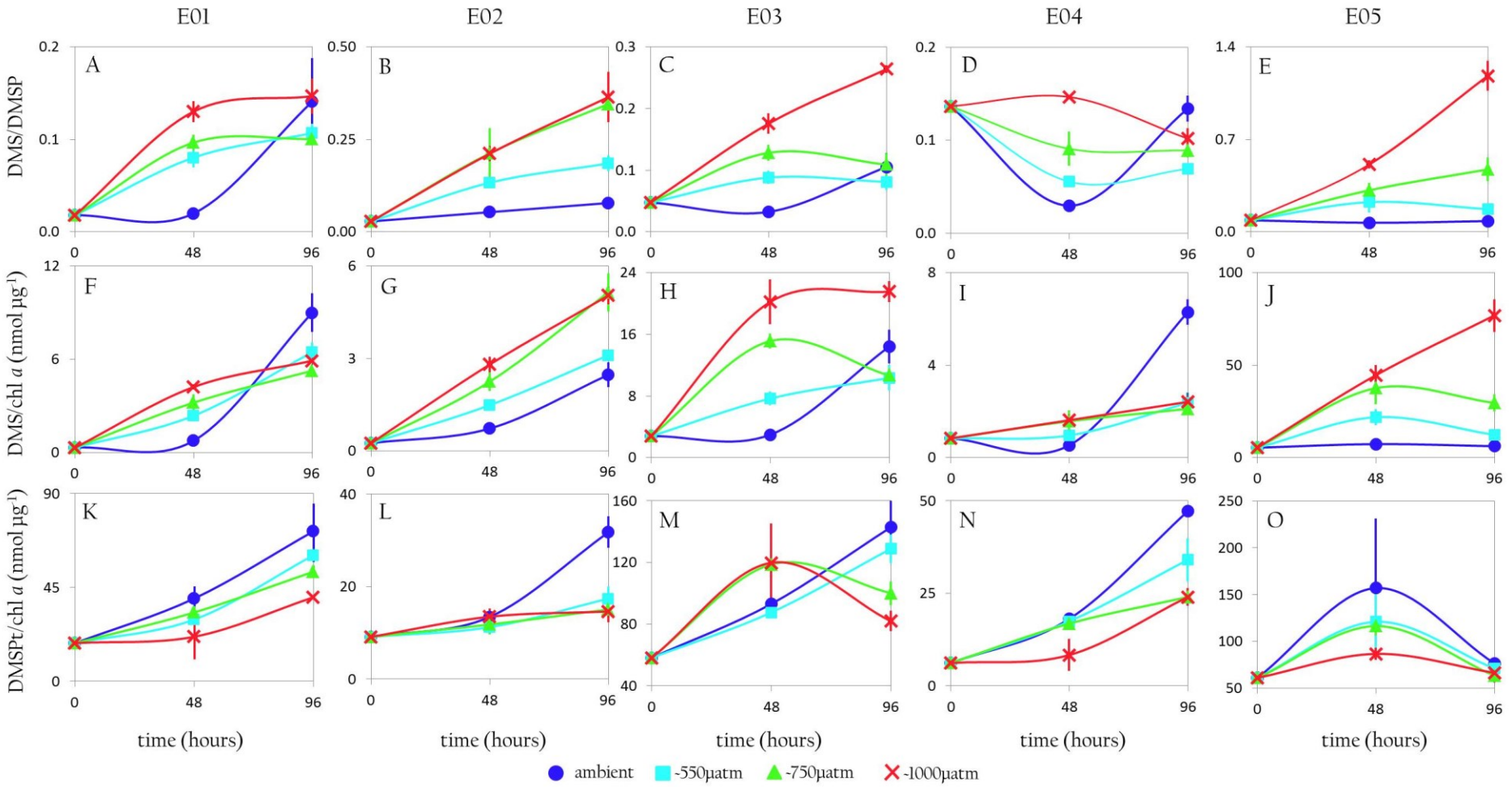

Figure S3. Ratios of DMS to DMSPt (A – E), DMS to Chl *a* (nmol µg^{-1}) (F – J) and DMSPt to Chl *a* (nmol µg^{-1}) (K – O) during five bioassay experiments. Values shown are means of experimental triplicates, and error bars indicate the standard error.