The following supplement accompanies the article

Response of benthic assemblages to multiple stressors: comparative effects of nutrient enrichment and physical disturbance

Joseph M. Kenworthy*, David M. Paterson, Melanie J. Bishop

*Corresponding author: joseph.kenworthy@sb-roscoff.fr

Marine Ecology Progress Series 532: 37–51 (2016)

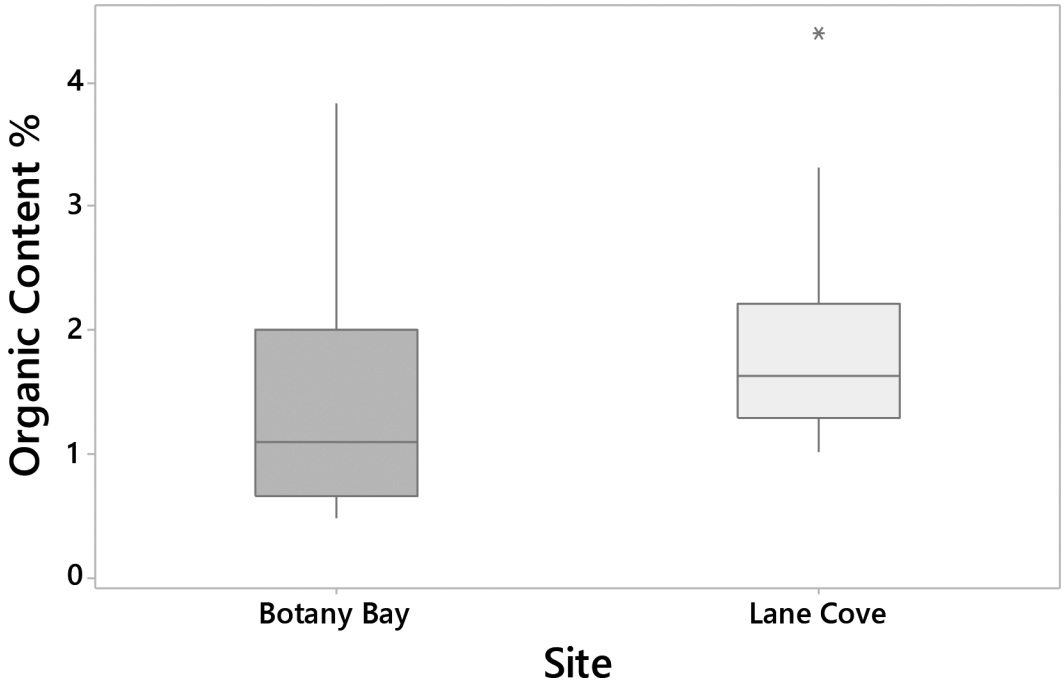

Figure S1: Sediment organic content (%) at the start of the experiment at sites in Botany Bay (mean = 1.49, median = 1.08, SD = 1.01, IQR = 0.65-2.00, n = 20) and Lane Cove (mean = 1.82, median = 1.62, SD = 0.79, IQR = 1.28-2.21, n = 25).

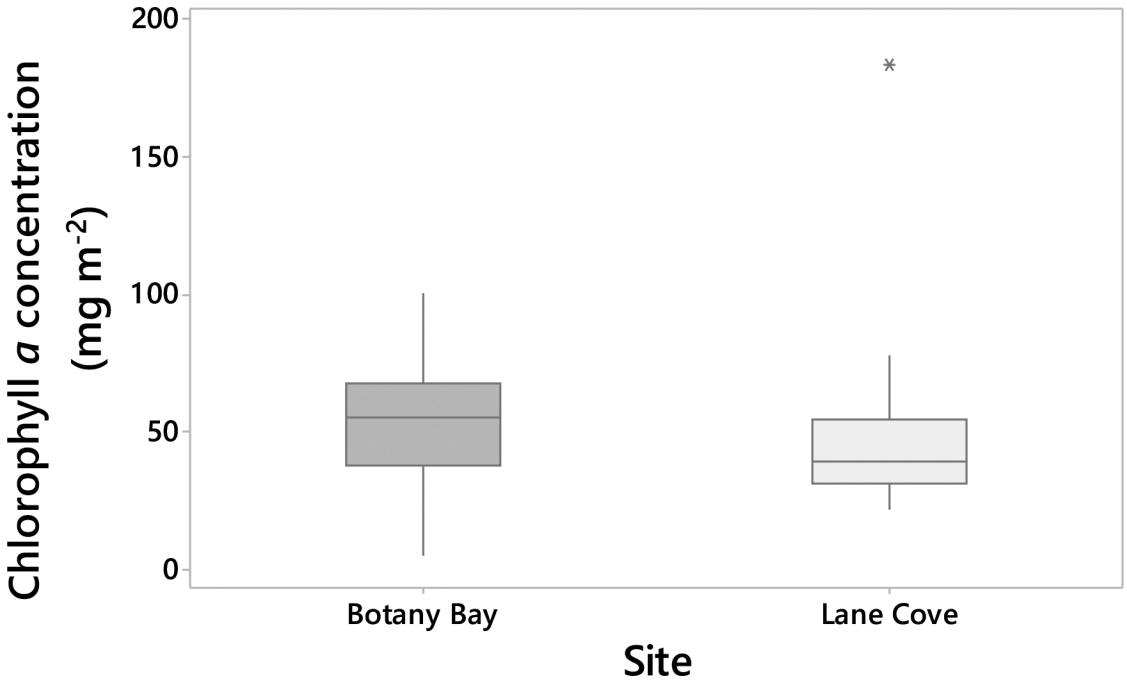

Figure S2: Sediment chlorophyll concentration (mg m^{-2}) at the start of the experiment at sites in Botany Bay (mean = 51.96, median = 55.10, SD = 22.44, IQR = 37.44-67.44, n = 25) and Lane Cove (mean = 47.12, median = 39.07, SD = 31.48, IQR = 31.07-54.07, n = 25).

Table S1: Descriptive statistics (means, standard errors [SE]) examining pre-existing differences in Normalised Difference Vegetation Index (NDVI) between sites (Botany Bay and Lane Cove) and treatment assignments , at the start of the experiment, prior to the application of stressors. For each of the two stressors, nutrient enrichment (N), and physical disturbance (D), plots were assigned to one of three levels (0 = Zero, L = Low, H = High), n = 5-7 for all treatments.

	Botany Bay			Lane Cove		
Treatment	**N**	**Mean**	**SE**	**n**	**Mean**	**SE**
0N0D	5	0.073	0.009	7	0.102	0.010
0NLD	6	0.064	0.002	7	0.081	0.009
0NHD	6	0.082	0.010	6	0.132	0.015
LN0D	5	0.069	0.005	6	0.094	0.010
LNLD	6	0.073	0.008	8	0.099	0.009
LNHD	6	0.087	0.007	6	0.095	0.010
HN0D	7	0.074	0.006	7	0.113	0.013
HNLD	5	0.073	0.007	7	0.106	0.015
HNHD	7	0.075	0.007	7	0.093	0.012
Site	**53**	**0.075**	**0.002**	**61**	**0.101**	**0.004**

Table S2: PERMANOVA analysis testing for pre-existing differences between sites and among treatment assignments in Normalised Difference Vegetation Index (NDVI) and macrofaunal abundance, species richness, Shannon diversity and multivariate community structure. Plots at each of two sites (S; Botany Bay, Lane Cove) were assigned to one of 3 levels (zero, low high) of each of the two stressors, nutrient enrichment (N) and physical disturbance (D), with n=5-7 plots per treatment per site. Pre-existing differences in chlorophyll *a* concentration and organic content among treatment assignments could not be tested as these were only sampled in a subset of plots prior to the manipulations.

	NDVI			**Abundance**			**Species Richness**		**Shannon**		**Multivariate**	
Factor	**df**	**Pseudo-*F***	**P(perm)**	**df**	**Pseudo-*F***	**P(perm)**	**Pseudo-*F***	**P(perm)**	**Pseudo-F**	**P(perm)**	**Pseudo-*F***	**P(perm)**
N	2	0.13	0.872	2	0.53	0.709	1.97	0.300	0.96	0.519	0.87	0.532
D	2	1.45	0.242	2	0.61	0.625	0.93	0.501	0.05	0.947	0.85	0.574
S	1	**30.95**	**<0.001**	1	**3.70**	**0.031**	0.12	0.723	0.43	0.510	**31.63**	**0.001**
NxD	4	2.00	0.104	4	2.44	0.184	0.16	0.959	1.00	0.492	0.67	0.824
NxS	2	0.40	0.671	2	1.94	0.124	2.33	0.118	1.14	0.352	1.16	0.284
DxS	2	0.36	0.693	2	0.06	0.939	0.49	0.621	0.39	0.691	0.95	0.543
NxDxS	4	0.66	0.622	4	0.39	0.822	2.46	0.061	0.41	0.806	0.98	0.510
Residuals	95			108								

Key: **Bold** = significant effect at $p < 0.05$

Table S3: PERMANOVA analysis testing for experimental artefacts associated with the burial of fertiliser at Botany Bay and Lane Cove estuaries on organic content, microphytobenthos variables (chlorophyll *a* concentration, Normalised Difference Vegetation Index [NDVI]) and macrofaunal variables (abundance, species richness, Shannon diversity and multivariate community analysis). Analyses examined the interacting effects of treatment (T; fixed two levels: control and procedural control), Month (M; random; 4 levels for microphytobenthos variables; 2 levels for macrofaunal variables) and Site (S; random). Plot (P) was nested within TxS. N = 3-7 for all treatment levels.

Organic content				**Chlorophyll *a***				**NDVI**	
Source	**df**	**Pseudo-*F***	**P(perm)**	**Df**	**Pseudo-*F***	**P(perm)**	**df**	**Pseudo-*F***	**P(perm)**
T	1	4.11	0.126	1	2.01	0.291	1	0.14	0.943
M	3	0.95	0.519	3	1.70	0.307	3	0.61	0.668
S	1	**4.22**	**0.027**	1	0.06	1.000	1	**5.56**	**0.030**
TxM	3	0.66	0.589	3	4.05	0.138	3	0.45	0.747
TxS	1	0.17	0.988	1	0.28	0.934	1	1.82	0.166
MxS	3	0.82	0.507	3	**8.96**	**0.001**	3	**4.46**	**0.007**
P(TxS)	24	**2.66**	**0.027**	24	**5.01**	**0.001**	23	1.35	0.181
TxMxS	3	1.90	0.163	3	0.41	0.749	3	0.29	0.826
Residuals	46			44			59		

		Abundance		**Species Richness**		**Shannon**		**Multivariate**	
Source	**df**	**Pseudo-*F***	**P(perm)**	**Pseudo-*F***	**P(perm)**	**Pseudo-*F***	**P(perm)**	**Pseudo-*F***	**P(perm)**
T	1	0.25	0.748	0.62	0.635	0.72	0.765	1.28	0.302
M	1	0.01	0.810	3.74	0.483	3.98	0.519	1.02	0.498
S	1	2.27	0.153	**7.11**	**0.018**	3.31	0.076	**6.44**	**0.001**
TxM	1	6.86	0.293	0.56	0.650	0.15	0.769	0.86	0.637
TxS	1	0.15	0.985	0.63	0.724	0.53	0.807	1.01	0.480
MxS	1	4.47	0.051	**5.04**	**0.041**	6.29	0.020	**3.58**	**0.001**
P(TxS)	24	6.93	0.001	**3.09**	**0.004**	3.90	0.002	**1.99**	**0.001**
TxMxS	1	0.12	0.729	1.43	0.256	1.41	0.220	0.61	0.815
Residuals	22								

Table S4: Two-way fully orthogonal PERMANOVAs examining the interacting effects of Nutrients (N) and Disturbance (D) on taxa identified by SIMPER analysis as key contributors (dissimilarity to standard deviation ratio > 1) to multivariate differences among treatments at 1 of the 2 sites. Analyses were performed separately for the the two sites, Botany Bay and Lane Cove, and used square root transformed abundances of taxa after 4 months of stressor application. There were three levels of each of nutrient enrichment and disturbance (zero, low, high). N=7 for all treatments.

		Botany Bay		**Lane Cove**	
Species	**Factor**	**F**	**P**	**F**	**P**
Salinator fragilis	**N**	0.71	0.494	-	-
	D	1.03	0.363	-	-
	NxD	0.87	0.485	-	-
Owenia australis	**N**	0.08	0.926	-	-
	D	0.08	0.911	-	-
	NxD	0.94	0.436	-	-
***Scoloplos* sp.**	**N**	-	-	**5.25**	**0.006**
	D	-	-	1.13	0.329
	NxD	-	-	**2.91**	**0.039**
Nematodes	**N**	-	-	**3.25**	**0.045**
	D	-	-	0.31	0.751
	NxD	-	-	1.17	0.331
***Gammarus* sp. 1**	**N**	-	-	0.21	0.809
	D	-	-	0.99	0.384
	NxD	-	-	2.11	0.094
Oedicerotidae	**N**	-	-	2.32	0.094
	D	-	-	0.99	0.385
	NxD	-	-	1.33	0.249
Platynerus uniseris	**N**	-	-	0.53	0.567
	D	-	-	0.03	0.980
	NxD	-	-	0.98	0.406
***Gammarus* sp. 2**	**N**	-	-	1.18	0.301
	D	-	-	0.21	0.823
	NxD	-	-	1.68	0.177
Australonereis ehlersi	**N**	-	-	1.31	0.265
	D	-	-	1.65	0.201
	NxD	-	-	1.09	0.385
***Laternula* sp.**	**N**	-	-	0.97	0.405
	D	-	-	0.31	0.725
	NxD	-	-	1.61	0.174

Key: **Bold** = significant effect at $p < 0.05$