

# Behavioural and physiological responses to pharmaceutical exposure in macroalgae and grazers from a Baltic Sea littoral community

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**Supplement 1.** Results from the statistical analyses examining the effects of 2 pharmaceuticals, ibuprofen and propranolol, on *Gammarus* spp. and *Fucus vesiculosus*. Ibuprofen resulted in no significant effects. PL/PM/PH: propranolol at 100, 1000 and 5000  $\mu\text{g l}^{-1}$ , respectively; Dose resp: dose response relationship, ns: non-significant. The figures represent main effects and comparison with control data obtained from the differences of least squares means (SAS v. 9.2). Corrected: multiplicity corrections according to Holm (1979).

Table S1. (A) Expt C, activity measured in the Multispecies Freshwater Biomonitor™. (B) Comparisons between treatments and control

## A

Effect	Num df	Den df	<i>F</i>	p
Concentration	0.1654	0.1712	49	<0.0001
Predator	-0.3367	0.2422	49	0.0164
Concentration × Predator	-0.1712	0.2422	49	ns

## B

Treatment	Treatment	Estimate	Error	df	<i>t</i>	p	Corrected
PL	Control	8.28	0.2966	39	2.36	0.02	Significant
PM	Control	8.12	0.2966	39	2.34	0.03	Significant
PH	Control		0.2966	39	8.64	<0.0001	Significant

Table S2. *Gammarus* spp. (A) Expt B, predator recognition. (B) Comparisons between treatments and control

**A**

Comparison	Estimate	Error	df	<i>t</i>	p	Corrected
Propranolol-Ibuprofen	-0.1250	0.2542	49	-0.49	ns	ns
Propranolol- Control	-0.1250	0.3595	49	-0.35	ns	ns
Dose resp propranolol	-1	0.4403	49	-2.27	0.028	ns

**B**

Treatment	Treatment	Estimate	Error	df	<i>t</i>	p	Corrected
PL	Control	0.3750	0.4403	49	0.85	ns	ns
PM	Control	-0.1250	0.4403	49	-0.28	ns	ns
PH	Control	-0.6250	0.4403	49	-1.42	ns	ns

Table S3. *Gammarus* spp. (A) Expt C, habitat search time. (B) Comparisons between treatments and control

**A**

Comparison	Estimate	Error	df	<i>t</i>	p	Corrected
Dose resp propranolol	68.25	20.41	36	3.34	0.002	Significant

**B**

Treatment	Treatment	Estimate	Error	df	<i>t</i>	p	Corrected
PL	Control	-10.77	18.74	36	-0.57	ns	ns
PM	Control	-44.42	17.82	36	-2.49	0.02	Significant
PH	Control	-51.42	18.24	36	-2.82	0.008	Significant

Table S4. *Gammarus* spp. (A) Expt A, feeding rate. (B) Comparisons between treatments and control

**A**

Comparison	Estimate	Error	df	<i>t</i>	p	Corrected
Propranolol-Ibuprofen	-0.6045	0.1371	55	-4.41	<0.0001	Significant
Propranolol- Control	0.6300	0.1919	55	3.28	0.0018	Significant

**B**

Treatment	Treatment	Estimate	Error	df	<i>t</i>	p	Corrected
PL	Control	0.6700	0.2350	55	2.85	0.006	Significant
PM	Control	0.7122	0.2350	55	3.03	0.0037	Significant
PH	Control	0.5078	0.2350	55	2.16	0.035	ns

Table S5. *Gammarus* spp. (A) Expt B, respiration. (B) Comparisons between treatments and control

**A**

Comparison	Estimate	Error	df	<i>t</i>	p	Corrected
Propranolol-Ibuprofen	-0.00125	0.002077	49	-0.60	ns	ns
Propranolol- Control	0.00042	0.002938	49	0.14	ns	ns
Dose-resp propranolol	0.0100	0.003598	49	2.78	0.008	Significant

**B**

Treatment	Treatment	Estimate	Error	df	<i>t</i>	p	Corrected
PL	Control	-0.00500	0.003598	49	-1.39	ns	ns
PM	Control	0.001250	0.003598	49	0.35	ns	ns
PH	Control	0.005000	0.003598	49	1.39	ns	ns

Table S6. *Fucus vesiculosus*. (A) Expt B, chlorophyll fluorescence. (B) Comparisons between treatments and control

**A**

Comparison	Estimate	Error	df	<i>t</i>	p	Corrected
Propranolol-Ibuprofen	0.1300	0.02345	48	5.54	<0.0001	Significant
Propranolol-Control	-0.12.52	0.03489	48	-3.59	0.0008	Significant
Does resp propranolol	-0.39	0.04061	48	-9.60	<0.0001	ns

**B**

Treatment	Treatment	Estimate	Error	df	<i>t</i>	p	Corrected
PL	Control	0.01393	0.04204	48	0.33	ns	ns
PM	Control	-0.01357	0.04204	48	-0.32	ns	ns
PH	Control	-0.3761	0.04204	48	-8.95	<0.0001	Significant

Table S7. *Fucus vesiculosus*. (A) Expt B, gross production to respiration ratio. (B) Comparisons between treatments and control

**A**

Comparison	Estimate	Error	df	<i>t</i>	p	Corrected
Propranolol-Ibuprofen	4.3692	1.2222	49	3.57	0.0008	Significant
Propranolol- Control	-1.9892	1.7285	49	-1.15	ns	ns
Dose resp propranolol	-7.8263	2.1170	49	-3.70	0.0006	Significant

**B**

Treatment	Treatment	Estimate	Error	df	<i>t</i>	p	Corrected
PL	Control	1.5575	2.1170	49	0.74	ns	ns
PM	Control	-1.2563	2.1170	49	-0.59	ns	ns
PH	Control	-6.2688	2.1170	49	-2.96	0.0047	Significant

Table S8. *Fucus vesiculosus*. Expt B, respiration. (B) Comparisons between treatments and control

**A**

Comparison	Estimate	Error	df	<i>t</i>	p	Corrected
Propranolol-Ibuprofen	-0.11171	0.03803	49	-3.08	0.003	Significant
Propranolol- Control	0.03875	0.05378	49	0.72	ns	ns
Dose resp propranolol	0.2900	0.06587	49	4.40	<0.0001	Significant

**B**

Treatment	Treatment	Estimate	Error	df	<i>t</i>	p	Corrected
PL	Control	-0.07125	0.06587	49	-1.08	ns	ns
PM	Control	-0.03125	0.06587	49	-0.47	ns	ns
PH	Control	0.2188	0.06587	49	3.32	0.0017	Significant

Table S9. *Fucus vesiculosus*. (A) Expt B, gross production. (B) Comparisons between treatments and control  
**A**

Comparison	Estimate	Error	df	<i>t</i>	p	Corrected
Propranolol-Ibuprofen	0.9533	0.2333	49	4.09	0.0002	Significant
Propranolol- Control	-0.6087	0.3300	49	-1.84	ns	ns
Dose response propranolol	-2.0063	0.4041	49	-4.96	<0.0001	Significant

**B**

Treatment	Treatment	Estimate	Error	df	<i>t</i>	p	Corrected
PL	Control	0.2600	0.4041	49	0.64	ns	ns
PM	Control	-0.3400	0.4041	49	-0.84	ns	ns
PH	Control	-1.7462	0.4041	49	-4.32	<0.0001	Significant

**Supplement 2.** After exposure to propranolol or ibuprofen in 3 different concentrations, there was a linear relationship between respiration and feeding rate for *Gammarus* spp., with the exception of one outlier (PL treatment).

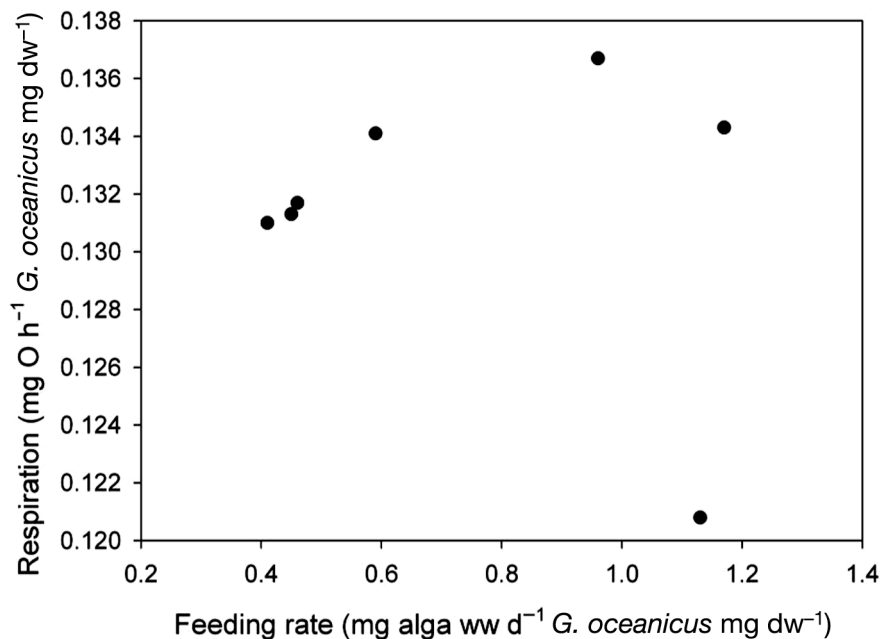


Fig. S1. *Gammarus* spp. Relation between feeding rate and respiration in *Gammarus* spp. exposed to 3 concentrations of propranolol and ibuprofen. ww: wet weight; dw: dry weight

LITERATURE CITED

Holm S (1979) A simple sequential rejective multiple test procedure. Scand J Stat 6:65–70