

Supplementary material

**Bioaccumulation, oxidative stress and cellular damage in the intertidal gastropod *Bembicium nanum* exposed to a metal contamination gradient**

Rodney P. Ubrihien<sup>A,B</sup>, Anne M. Taylor<sup>A</sup> and William A. Maher<sup>A</sup>

<sup>A</sup>Ecochemistry Laboratory, Institute for Applied Ecology, University of Canberra, University Drive, Bruce, ACT 2617, Australia.

<sup>B</sup>Corresponding author. Email: rod.ubrihien@canberra.edu.au

**Table S1. Measured values and certified reference values ( $\mu\text{g g}^{-1}$  dry mass) for certified reference material 1556b samples that were digested and analysed with metal samples**

Element	Measured value	Certified value
Mn	17 ± 1	18.5 ± 0.2
Fe	178 ± 15	206 ± 7
Cu	66 ± 5	72 ± 2
Zn	1285 ± 122	1424 ± 46
As	7.1 ± 0.4	7.65 ± 0.65
Cd	2.4 ± 0.3	2.48 ± 0.08

**Table S2. Metal concentrations ( $\mu\text{g g}^{-1}$  dry mass) in *B. nanum* whole tissue for organisms sampled at Port Kembla, Shellharbour and Kiama**

Mean and standard error values are calculated from combined data for organisms sampled September 2010, November 2011, January 2012 and March 2012 ( $n = 22$ ) (% mol indicates the mean percentage of the total molar concentration of the metal burden for the site)

	Port Kembla		Shellharbour		Kiama	
	Mean ± s.e.	% mol	Mean ± s.e.	% mol	Mean ± s.e.	% mol
Mn	86 ± 10	6.6	59 ± 13	11.8	41 ± 8	8.9
Fe	351 ± 38	26.5	251 ± 32	48.9	169 ± 20	36.1
Cu	839 ± 83	55.7	71 ± 11	12.1	152 ± 23	28.5
Zn	148 ± 10	9.5	128 ± 6	21.2	125 ± 11	22.8
As	30 ± 4	1.7	40 ± 9	5.9	22 ± 2	3.5
Cd	1.21 ± 0.24	0	1.61 ± 0.25	0.1	1.75 ± 0.24	0.2

**Table S3. Mann–Whitney U-tests comparing concentrations of Fe, Cu, Zn, As, Mn and Cd in digestive tracts and mantle complex tissue of *B. nanum* sampled from Port Kembla, Shellharbour and Kiama**

Data used are from combined replicates from November 2011, January 2012 and March 2012 ( $n = 12$ )

	Port Kembla			Shellharbour			Kiama		
	<i>U</i>	<i>Z</i>	<i>P</i>	<i>U</i>	<i>Z</i>	<i>P</i>	<i>U</i>	<i>Z</i>	<i>P</i>
Fe	12	-3.464	0.001	27	-2.598	0.005	58	-0.808	0.210
Cu	3	-3.984	0.000	67	-0.289	0.386	69	-0.173	0.432
Zn	0	-4.157	0.000	0	-4.157	0.000	12	-3.464	0.001
As	5	-3.868	0.000	11	-3.522	0.000	5	-3.868	0.000
Mn	0	-4.157	0.000	0	-4.157	0.000	0	-4.157	0.000
Cd	60	-0.693	0.244	45	-1.559	0.060	65	-4.04	0.343

**Table S4. Manganese concentration in digestive tract (dig) and mantle complex tissue (tis) of *B. nanum* sampled from Port Kembla, Shellharbour and Kiama (all is the three sites combined)**

Data are for combined replicates from November 2011, January 2012 and March 2012 ( $n = 12$ )

	Mean $\pm$ s.e.	Min.	Max.
Mn <sub>dig</sub> (All)	213 $\pm$ 139	26	486
Mn <sub>tis</sub> (All)	10 $\pm$ 6	3	33
Mn <sub>whole</sub> (All)	79 $\pm$ 49	11	190
Mn <sub>dig</sub> (PK)	284 $\pm$ 124	143	486
Mn <sub>tis</sub> (PK)	12 $\pm$ 8	5	33
Mn <sub>whole</sub> (PK)	100 $\pm$ 41	48	190
Mn <sub>dig</sub> (S)	200 $\pm$ 157	26	452
Mn <sub>tis</sub> (S)	8 $\pm$ 3	3	14
Mn <sub>whole</sub> (S)	76 $\pm$ 57	11	181
Mn <sub>dig</sub> (K)	156 $\pm$ 112	56	393
Mn <sub>tis</sub> (K)	11 $\pm$ 6	4	29
Mn <sub>whole</sub> (K)	59 $\pm$ 40	24	142

**Table S5. Arsenic concentration in digestive tract (dig) and mantle complex tissue (tis) of *B. nanum* sampled from Port Kembla, Shellharbour and Kiama (all is the three sites combined)**

Data are for combined replicates from November 2011, January 2012 and March 2012 ( $n = 12$ )

	Mean $\pm$ s.e.	Min.	Max.
AS <sub>dig</sub> (All)	47 $\pm$ 46	19	267
AS <sub>tis</sub> (All)	18 $\pm$ 5	10	30
AS <sub>whole</sub> (All)	28 $\pm$ 16	14	93
AS <sub>dig</sub> (PK)	60 $\pm$ 66	20	267
AS <sub>tis</sub> (PK)	20 $\pm$ 6	13	30
AS <sub>whole</sub> (PK)	33 $\pm$ 20	17	93
AS <sub>dig</sub> (S)	42 $\pm$ 41	19	168
AS <sub>tis</sub> (S)	19 $\pm$ 4	12	25
AS <sub>whole</sub> (S)	28 $\pm$ 17	16	80
AS <sub>dig</sub> (K)	37 $\pm$ 21	19	83
AS <sub>tis</sub> (K)	15 $\pm$ 4	10	24
AS <sub>whole</sub> (K)	23 $\pm$ 9	14	44

**Table S6. Iron Concentration in digestive tract (dig) and mantle complex tissue (tis) of *B. nanum* sampled from Port Kembla, Shellharbour and Kiama (all is the three sites combined)**

Data are for combined replicates from November 2011, January 2012 and March 2012 ( $n = 12$ )

	Mean $\pm$ s.e.	Min.	Max.
Fe <sub>dig</sub> (All)	456 $\pm$ 276	96	1455
Fe <sub>tis</sub> (All)	239 $\pm$ 109	74	491
Fe <sub>whole</sub> (All)	313 $\pm$ 151	106	853
Fe <sub>dig</sub> (PK)	648 $\pm$ 293	365	1455
Fe <sub>tis</sub> (PK)	285 $\pm$ 124	145	491
Fe <sub>whole</sub> (PK)	405 $\pm$ 176	215	853
Fe <sub>dig</sub> (S)	466 $\pm$ 241	169	879
Fe <sub>tis</sub> (S)	221 $\pm$ 110	73	434
Fe <sub>whole</sub> (S)	307 $\pm$ 131	141	572
Fe <sub>dig</sub> (K)	255 $\pm$ 118	96	417
Fe <sub>tis</sub> (K)	212 $\pm$ 82	105	355
Fe <sub>whole</sub> (K)	228 $\pm$ 85	106	345

**Table S7. Zinc concentration in digestive tract (dig) and mantle complex tissue (tis) of *B. nanum* sampled from Port Kembla, Shellharbour and Kiama (all is the three sites combined)**

Data are for combined replicates from November 2011, January 2012 and March 2012 ( $n = 12$ )

	Mean $\pm$ s.e.	Min	Max
Zn <sub>dig</sub> (All)	242 $\pm$ 134	100	730
Zn <sub>tis</sub> (All)	77 $\pm$ 42	50	312
Zn <sub>whole</sub> (All)	131 $\pm$ 49	76	293
Zn <sub>dig</sub> (PK)	325 $\pm$ 195	156	730
Zn <sub>tis</sub> (PK)	72 $\pm$ 14	57	99
Zn <sub>whole</sub> (PK)	150 $\pm$ 57	95	284
Zn <sub>dig</sub> (S)	238 $\pm$ 49	158	311
Zn <sub>tis</sub> (S)	66 $\pm$ 11	50	86
Zn <sub>whole</sub> (S)	126 $\pm$ 19	95	152
Zn <sub>dig</sub> (K)	163 $\pm$ 51	99	252
Zn <sub>tis</sub> (K)	93 $\pm$ 69	62	311
Zn <sub>whole</sub> (K)	116 $\pm$ 58	76	293

**Table S8. Copper concentration in digestive tract (dig) and mantle complex tissue (tis) of *B. nanum* sampled from Port Kembla, Shellharbour and Kiama (all is the three sites combined)**

Data are for combined replicates from November 2011, January 2012 and March 2012 ( $n = 12$ )

	Mean $\pm$ s.e.	Min.	Max.
Cu <sub>dig</sub> (All)	612 $\pm$ 765	7	2770
Cu <sub>tis</sub> (All)	211 $\pm$ 220	31	881
Cu <sub>whole</sub> (All)	341 $\pm$ 390	26	1609
Cu <sub>dig</sub> (PK)	1518 $\pm$ 619	729	2770
Cu <sub>tis</sub> (PK)	478 $\pm$ 178	218	881
Cu <sub>whole</sub> (PK)	812 $\pm$ 319	403	1609
Cu <sub>dig</sub> (S)	108 $\pm$ 95	19	338
Cu <sub>tis</sub> (S)	75 $\pm$ 32	31	149
Cu <sub>whole</sub> (S)	87 $\pm$ 52	32	217
Cu <sub>dig</sub> (K)	210 $\pm$ 347	7	1184
Cu <sub>tis</sub> (K)	79 $\pm$ 63	31	267
Cu <sub>whole</sub> (K)	122 $\pm$ 125	26	460