

Supplementary Material

Influence of calcium on the toxicity of saline solutions to the mayfly, *Austrophlebioides* sp. AV11

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Influence of calcium on the toxicity of saline solution to a species of mayfly, *Austrophlebioides* sp. AV11

This document consists of calcium proportion and 96-h LC_{50} values of previous studies, Cusum chart for the reference toxicant, graphs for the comparison of AMW with solutions with increased calcium concentrations, tables for ionic compositions and details of data about Mount Barney.

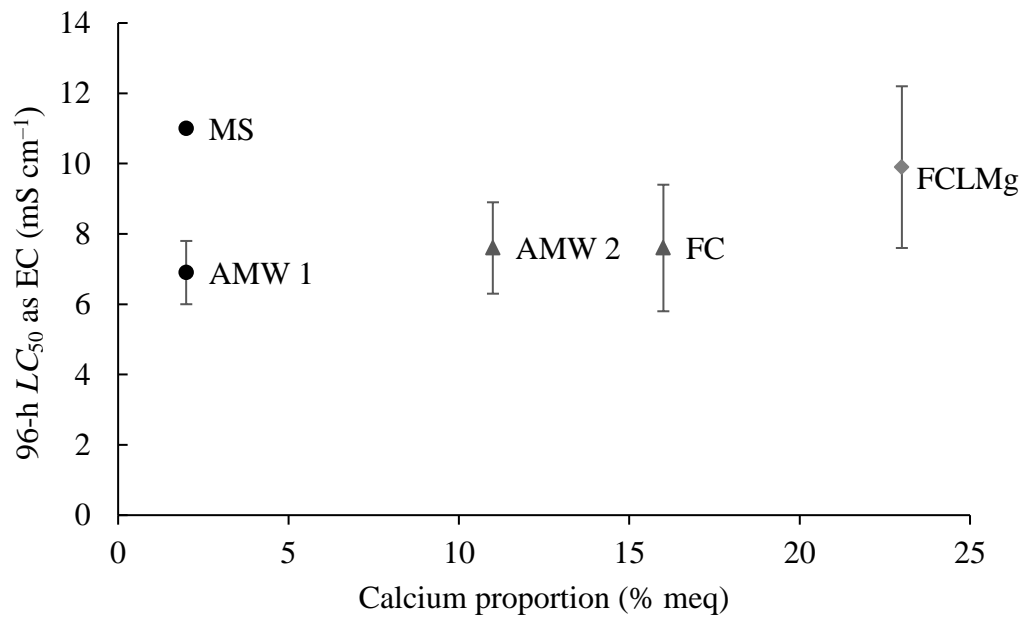


Figure S1. Concentration of calcium as proportion of major ions (% meq) against 96-h LC_{50} as electrical conductivity (EC, $mS\ cm^{-1}$) from studies of Dunlop *et al.* (2011) and Prasad *et al.* (2014). AMW 1 and AMW 2 are two compositions of artificial mine waters, FC is Fitzroy composition representing the composition of Fitzroy River, FCLMg is Fitzroy composition with low magnesium.

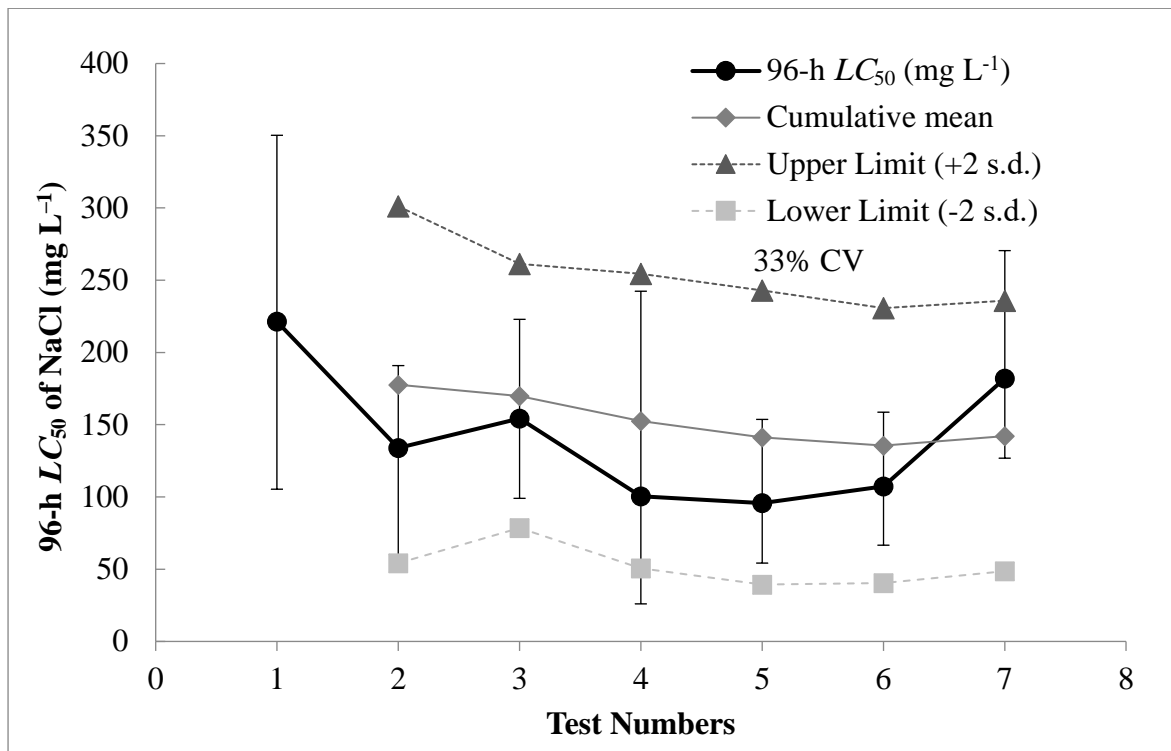


Figure S2. Cusum chart for reference toxicant NaCl showing the 96-h LC_{50} (mg L^{-1}) values with 95% CI (s.d.) for all tests, cumulative mean of 96-h LC_{50} , the upper limit, lower limit of two standard deviations and coefficient of variance (CV) of 33% (Environment Canada 1990; US Environmental Protection Agency 2002).

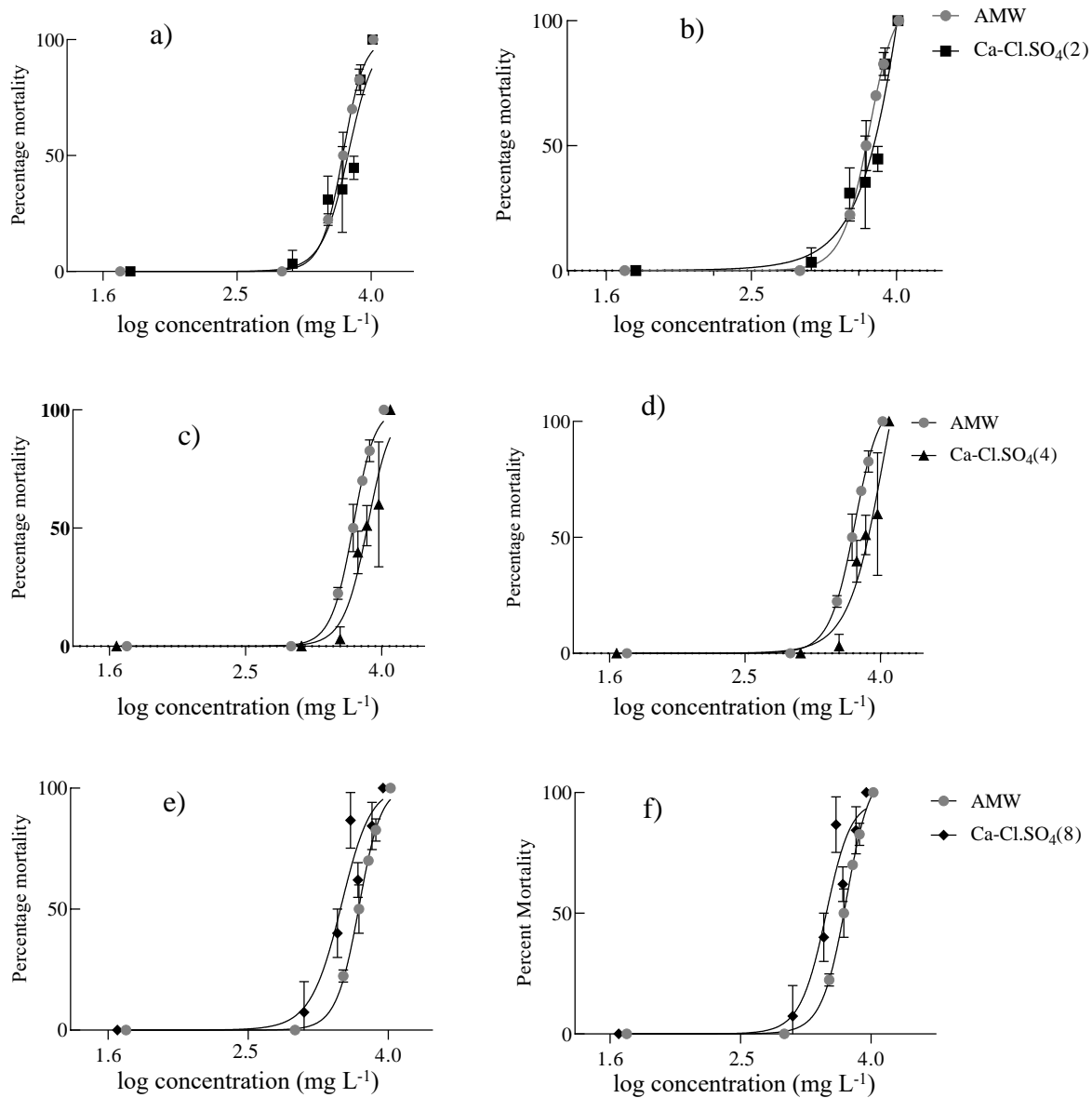


Figure S3. Comparison of percentage mortality between a) 96-h LC₅₀ for Ca-Cl.SO₄(2) and AMW; b) dose-response curve for Ca-Cl.SO₄(2) and AMW; c) 96-h LC₅₀ for Ca-Cl.SO₄(4) and AMW; d) dose-response curve for Ca-Cl.SO₄(4) and AMW ; e) 96-h LC₅₀ for Ca-Cl.SO₄(8) and AMW; f) dose-response curve for Ca-Cl.SO₄(8) and AMW; error bars represent 95% confidence intervals of standard deviations.

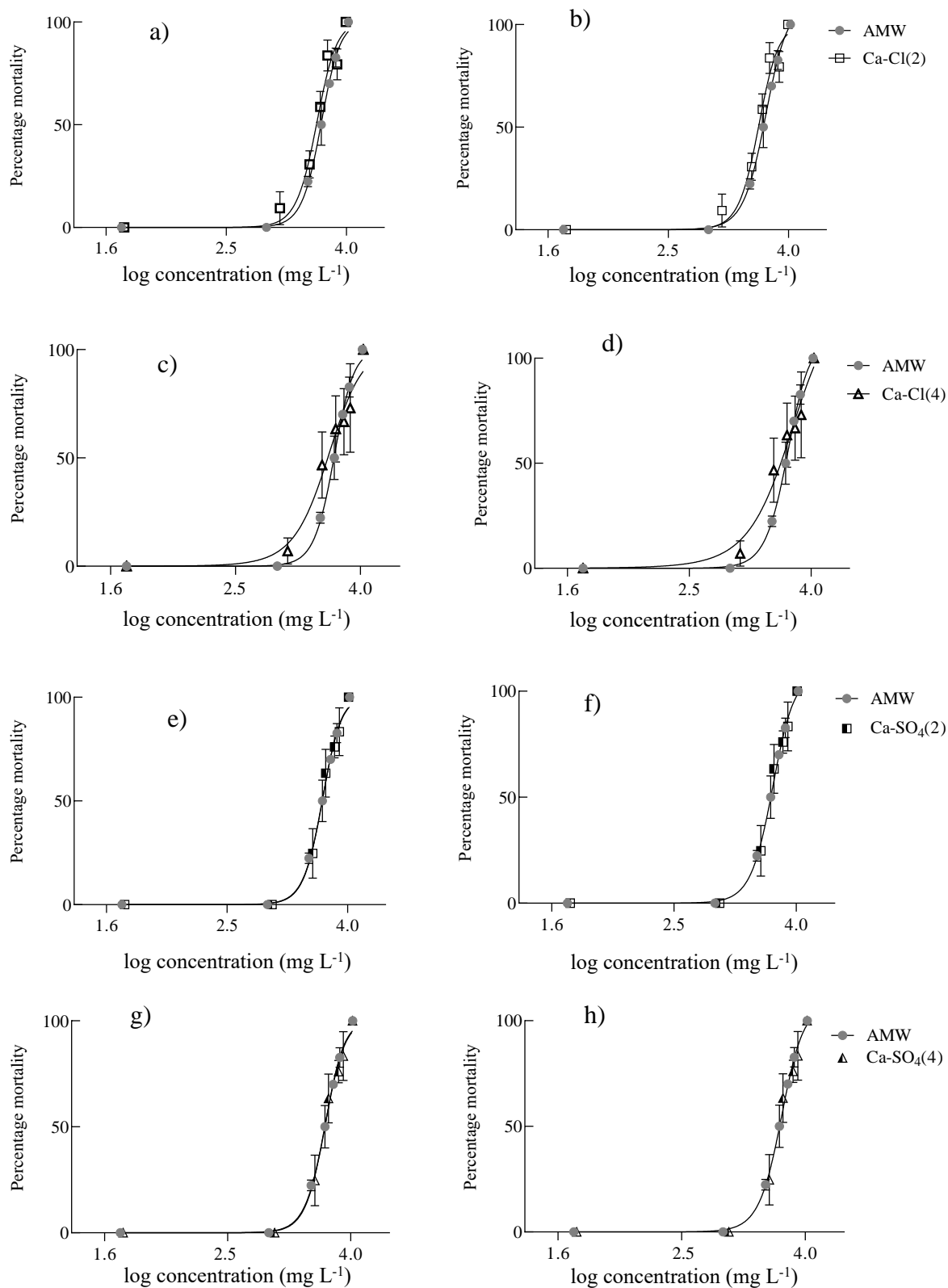


Figure S4. Comparison of percentage mortality between a) 96-h LC_{50} for Ca-Cl(2) and AMW, b) dose-response curve for Ca-Cl(2) and AMW; c) 96-h LC_{50} for Ca-Cl(4) and AMW, d) dose-response curve for Ca-Cl(4) and AMW; e) 96-h LC_{50} for Ca-SO₄(2) and AMW, f) dose-response curve for Ca-SO₄(2) and AMW; g) 96-h LC_{50} for Ca-SO₄(4) and AMW, h) dose-response curve for Ca-SO₄(4) and AMW; error bars represent 95% confidence intervals for standard deviations.

Tables showing the detail data for measured ionic composition of solutions (Table S1) and details of Mount Barney (Tables S2–S4).

Table S1. Dissolved oxygen (DO), temperature, electrical conductivity (EC) and pH for Mount Barney

Test ID	Collection date	DO (mg L ⁻¹)	Temperature (°C)	EC (μS cm ⁻¹)	pH
MB1	25/03/13	8.67	21.4	105.4	8.11
MB2	15/04/13	8.7	21.1	92.73	7.84
MB3	20/05/2013	9.34	12.9	55.3	7.87
MB4	5/06/2013	9.89	14.2	60.0	8.16
MB5	24/06/2013	9.84	13.5	Not recorded	Not recorded
MB6	5/08/2013	10.64	12.9	73.7	7.37
MB7	4/09/2013	9.14	18.1	95.65	8.54
MB8	23/09/2013	8.67	20.6	100.1	7.97
MB9	8/10/2013	8.26	22.6	106.3	8.14

Table S2. Measured ionic composition of stock solutions

Solution ID	Measured EC (mS cm ⁻¹)	Ca	K	Mg	Na	SO ₄	Cl	
		(mg L ⁻¹)						
AMW	15.1	161	53	410	3360	3160	4590	490
Ca-Cl.SO ₄ (2)	14.7	300	56	470	3310	3640	4240	430
Ca-Cl.SO ₄ (4)	18.5	430	48	410	2760	3230	4190	270
Ca-Cl.SO ₄ (8)	12.5	880	51	450	1870	2160	4240	150
Ca-Cl(2)	13.8	110	48	420	2830	3000	3830	440
Ca-Cl(4)	15.5	590	56	470	3210	3270	5150	250
Ca-SO ₄ (2)	14.5	250	54	390	3260	3610	4240	340
Ca-SO ₄ (4)	14.9	390	54	400	3300	4250	4280	200

Table S3. Concentration of major ions for Mount Barney creek during the collection of organisms for the experiments

Test ID	Test start date	Ca	K	Mg	Na	SO ₄	Cl	HCO ₃
		(mg L ⁻¹)						
MB1	26/03/2013	3.1	0.5	1.7	2.6	6.4	4.9	13.0
MB2	15/04/2013	3.0	0.4	1.6	1.8	1.8	9.3	Not analysed
MB3	21/05/2013	4.1	0.9	2.3	2.2	3.0	5.8	56.0
MB4	6/06/2013	4.3	0.6	2.4	1.1	2.5	7.0	62.9
MB5	25/06/2013	3.7	0.8	2.0	0.0	2.9	6.0	45.3
MB7	5/09/2013	4.5	0.8	2.5	1.9	3.1	7.0	42.1
MB8	24/09/2013	3.8	0.9	2.3	1.7	2.7	6.0	69.5
MB9	8/10/2013	5.2	1.0	2.9	2.8	2.9	7.0	59.8

Table S4. Descriptive statistics on stream water chemistry of Mount Barney creek

Description	Ca	K	Mg	Na	SO ₄	Cl	HCO ₃
	(mg L ⁻¹)						
Minimum	3.0	0.4	1.6	0.0	1.8	4.9	13.0
Maximum	5.2	1.0	2.9	2.8	6.4	9.3	69.5
Median	3.9	0.8	2.3	1.8	2.9	6.5	56.0
Mean	4.0	0.7	2.2	1.8	3.2	6.6	49.8
Standard Deviation	0.7	0.2	0.4	0.9	1.4	1.3	18.8
Standard Error of Mean	0.3	0.1	0.2	0.3	0.5	0.5	7.1
Number of samples	8	8	8	8	8	8	7

References

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