

The sub-lethal and reproductive effects of acute and chronic exposure to flowback and produced water from hydraulic fracturing on the water flea *Daphnia magna*

Tamzin A. Blewett^{1*}, Perrine L.M. Delompré^{1*}, Yuhe He¹, Erik J. Folkerts¹, Shannon L. Flynn²,
Daniel S. Alessi², Greg G. Goss^{1,3}

¹Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

²Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, Alberta, Canada

³National Institute for Nanotechnology, 11421 Saskatchewan Drive, Edmonton, Alberta, Canada

Corresponding author:
Tamzin Blewett
tamzin.blewett@ualberta.ca
Department of Biological Sciences
University of Alberta
Edmonton, AB
T6G 2R3

Supporting information (5 pages)

3 Tables : Exposure water chemistry and primer information

1 Figure : Relationship between oxygen consumption and neonate production

ICP QA/QC

Samples were analyzed as the average of 5 replicates each composed of 300 sweeps. After each run blank and standard counts were compared to previous run to ensure consistency and detect if

contamination had occurred. Instrumental drift was accounted for and corrected using an internal standard solution of 0.5 ppm indium solution in 2% nitric acid added to the sample using an online internal standard addition kit. After every 12 samples, blank and midrange standards were rerun to detect instrumental buildup of elements and as secondary check of instrumental drift. Replicate standards were found to vary by $\pm 4.9\%$ through duration of a run. All standard curves had $R^2 > .98$.

Table S1. Measured water chemistry (via ICP MS/MS) of FPW, control water, and estimated (Est.) elemental concentration in FPW if diluted in 18M Ω water, where TDS = total dissolved solids, TN = Total nitrogen TC= Total carbon. Reported values are means $\pm$ S.E.M, N=4.

	Undiluted	Control	Est. FPW 0.004%	Est. FPW 0.008%	Est. FPW 0.04%
	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
TDS	182533		7.30132	14.60264	73.0132
Na	59500	11.1 $\pm$ 2.3	2.38	4.76	23.8
Ca	6500	50.1 $\pm$ 2.5	0.26	0.52	2.6
K	2160	1.34 $\pm$ 0.2	0.0864	0.1728	0.864
Sr	931	0.5 $\pm$ 0.009	0.03724	0.07448	0.3724
Mg	706	13.9 $\pm$ 0.7	0.02824	0.05648	0.2824
Br	297	-	0.01188	0.02376	0.1188
B	96	0.01 $\pm$ 0.0006	0.00384	0.00768	0.0384
Li	51.3	BDL	0.002052	0.004104	0.02052
Mn	7.32	0.01 $\pm$ 0.0002	0.0002928	0.0005856	0.002928
Ba	7.28	BDL	0.0002912	0.0005824	0.002912
Zn	1.24	BDL	0.0000496	0.0000992	0.000496
Fe	1.19	-	0.0000476	0.0000952	0.000476
TC	737	-	0.02948	0.05896	0.2948
TN	425	-	0.017	0.034	0.17
Cl	107000	-	4.28	8.56	42.8

Table S3. Forward and reverse primers, and their efficiency (Ef), for assessed genes.

Gene	Abb	GeneBank	Forward primer	Reverse primer	Ef
prohibitin 2	phb2	DW724510	AATTGTTCAAGCCGAGG	CGTCAAAGGAAACGTCAC	2.03
carboxypeptidase A1 precursor	cpa1	DW724601	CCGACATTCACGTCAGT	CCTCGTAGGTGTGATAGTT	1.99
hemoglobin	hgb	DW724693	GGAAGCGGATTCACTG	TGTCACCCATAGCCGA	2.07
doublesex-Mab related 93B	dmrt93b	AB361070	CGACAACAAGCTACCGAAGA	GGTGAATCTCGTCCTCCAAATA	2.03
cuticle 12	cut	DW985490	AGCCAGTGGAACACTACG	TCCAGCATCATCAGCG	2.01
vitellogenin 1	vtg1	AB252737	GCTACCCACGTCAAGTAATG	GCTGCCGTAGTCTCAACAGAA	2.06
vitellogenin 2	vtg2	AB252738	GCACTCTTTCTCGTTATTGCTG	GATCTTGACTGGGCTATTGATT	2.00
ecdysone receptor a	ecr-a	EF363705	CAGTTCGTCCATGTCGATGAG	CGAAGGCGGTAAGGTAGAATG	2.05
ecdysone receptor b	ecr-b	AB274824	AGTCAGTTCGTCATCTTCTGTG	CACGTGACCGTAAGGGTATTT	2.00
ultraspiracle	usp	AB274819	TAGGCCACTCGGGTTACTTAAA	GAGTGGGTGGTTAGGTGGATAA	2.07
cytochrome P450 4	cyp4	AB257772	GCGGTCCTCAGTAGCAATAAA	GCTACCTGTGCTCGTCAATAG	2.04
cytochrome P450 314	cyp314	AB257771	AGAGCGTGTCATCGTAGAAAG	CAGGAACGTGATAGCCTTGAA	2.00
cytochrome P450 18a1	cyp18a1	AB839173	AGAACGTAGTCGAAGGTGAAAC	CTGCACTGGACAGCTCAAATA	1.90
glutathione-S-transferase	gst	KF736983	CGTCAATCTTATGGGAGGAGAAC	GAATCCCGAGTCATCCAAAGTAG	2.01
catalase	cat	GQ389639	CTGAAGGCAAACCTGTCTACT	CAGGATCATCGGCAGTTAGTT	2.10

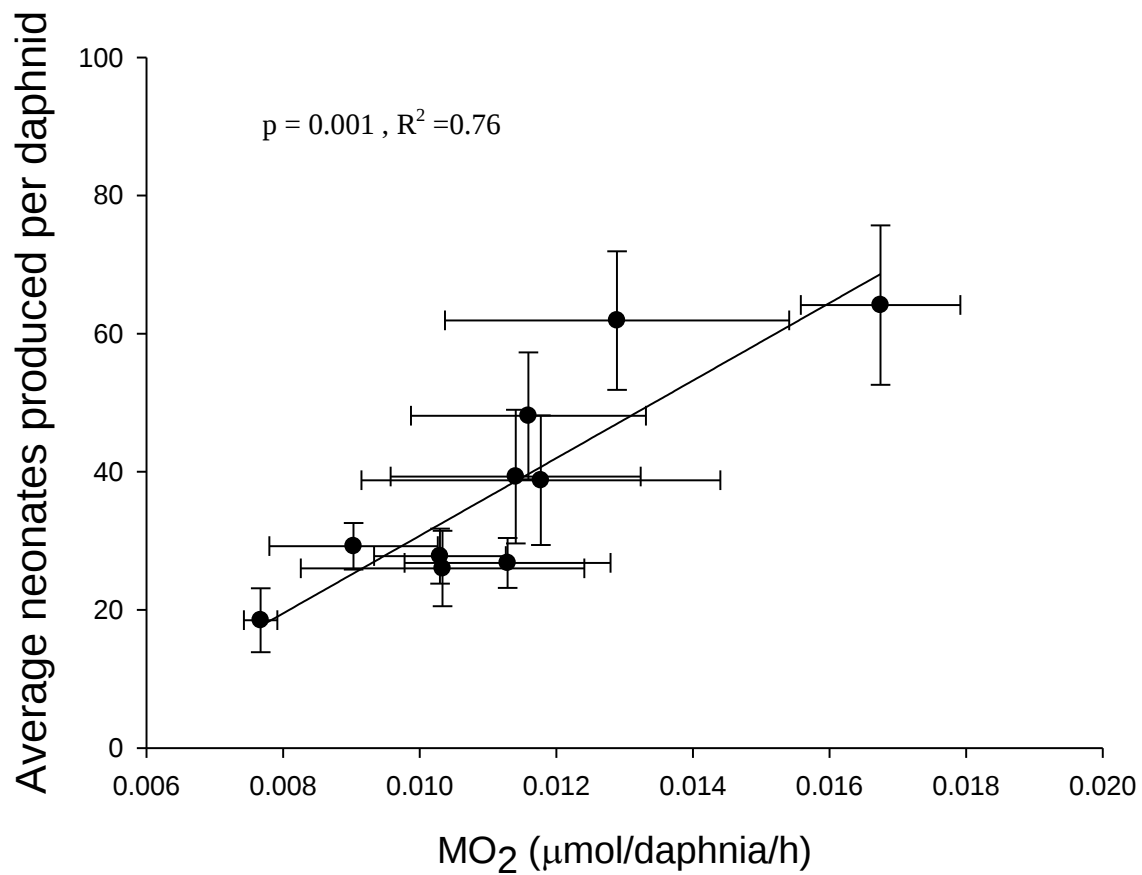


Figure S1. The average neonate production correlating with oxygen consumption in *Daphnia* ($\mu\text{mol/Daphnia/h}$), means are $\pm$ S.E.M, $p=0.001$, $R^2 = 0.76$