

SUPPLEMENTARY MATERIAL

Oxidative stress-related biomarkers in tissues of the euryhaline guppy *Poecilia vivipara* exposed *in situ* to a coastal water environment with long history of metal contamination

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Table S1. Comparison between the mean values of the physicochemical parameters of seawater (T: temperature; S: salinity; pH; O₂: dissolved oxygen content; and DOC: dissolved organic carbon) in the three exposure sites (Sites 1-3) of the euryhaline guppy *Poecilia vivipara* in a coastal water environment with long history of metal contamination. For each parameter, no significant difference was observed between the exposure sites (One-Way ANOVA; $p > 0.05$).

Univariate Tests of Significance for T (Water.sta) Sigma-restricted parameterization Effective hypothesis decomposition Include condition: v2=1					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	9987.556	1	9987.556	598.4554	0.000000
Site	10.111	2	5.056	0.3029	0.743065
Error	250.333	15	16.689		

Univariate Tests of Significance for S (Water.sta) Sigma-restricted parameterization Effective hypothesis decomposition Include condition: v2=1					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	16440.89	1	16440.89	742.8112	0.000000
Site	59.11	2	29.56	1.3353	0.292607
Error	332.00	15	22.13		

Univariate Tests of Significance for pH (Water.sta) Sigma-restricted parameterization Effective hypothesis decomposition Include condition: v2=1					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1148.802	1	1148.802	7727.369	0.000000
Site	0.068	2	0.034	0.228	0.798870
Error	2.230	15	0.149		

Univariate Tests of Significance for O ₂ (Water.sta) Sigma-restricted parameterization Effective hypothesis decomposition Include condition: v2=1					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	946.1250	1	946.1250	1901.971	0.000000
Site	0.3233	2	0.1617	0.325	0.727494
Error	7.4617	15	0.4974		

Univariate Tests of Significance for DOC (Water.sta) Sigma-restricted parameterization Effective hypothesis decomposition Include condition: v2=1					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	177.3472	1	177.3472	240.3441	0.000000
Site	1.6344	2	0.8172	1.1075	0.355940
Error	11.0683	15	0.7379		

Table S2. Comparison (Two-Way ANOVA followed by the Tukey HSD test) between mean concentrations of metals (Ag, Cd, Cr, Cu, Ni, Pb, and Zn) in tissues (1: gills; 2: liver) of the euryhaline guppy *Poecilia vivipara* exposed (96 h) to three different sites (Sites 1-3) in a coastal water environment with long history of metal contamination.

Univariate Tests of Significance for log Ag (Metals.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable log Ag (Metals.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .03512, df = 54.000				
Effect	SS	Degr. of Freedom	MS	F	p	Tissue	Site	log Ag Mean	1	2
Intercept	11.57220	1	11.57220	329.4914	0.000000	1	3	0.371068	****	
Tissue	0.27827	1	0.27827	7.9231	0.006797	1	2	0.371068	****	
Site	0.13349	2	0.06674	1.9004	0.159380	1	1	0.371068	****	
Tissue*Site	0.13349	2	0.06674	1.9004	0.159380	2	2	0.398034	****	****
Error	1.89655	54	0.03512			2	3	0.495564	****	****
						2	1	0.628216		****

Univariate Tests of Significance for Cd (Metals.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable Cd (Metals.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .00000, df = 54.000				
Effect	SS	Degr. of Freedom	MS	F	p	Tissue	Site	Cd Mean	1	
Intercept	54.13100	1	54.13100	32478601	0.000000	1	1	0.949000	****	
Tissue	0.00000	1	0.00000	1	0.321771	1	3	0.950000	****	
Site	0.00000	2	0.00000	1	0.374589	1	2	0.950000	****	
Tissue*Site	0.00000	2	0.00000	1	0.374589	2	1	0.950000	****	
Error	0.00009	54	0.00000			2	2	0.950000	****	
						2	3	0.950000	****	

Univariate Tests of Significance for log Cr (Metals.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable log Cr (Metals.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .05149, df = 54.000				
Effect	SS	Degr. of Freedom	MS	F	p	Tissue	Site	log Cr Mean	1	
Intercept	34.03145	1	34.03145	660.9835	0.000000	2	2	0.682915	****	
Tissue	0.00068	1	0.00068	0.0133	0.908702	1	3	0.744246	****	
Site	0.04636	2	0.02318	0.4503	0.639836	1	2	0.750918	****	
Tissue*Site	0.04470	2	0.02235	0.4341	0.650081	1	1	0.754073	****	
Error	2.78025	54	0.05149			2	3	0.771665	****	
						2	1	0.814908	****	

Univariate Tests of Significance for log Cu (Metals.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable log Cu (Metals.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .10389, df = 24.000				
Effect	SS	Degr. of Freedom	MS	F	p	Tissue	Site	log Cu Mean	1	2
Intercept	119.7992	1	119.7992	1153.105	0.000000	1	2	0.799399	****	
Tissue	37.2892	1	37.2892	358.921	0.000000	1	3	0.843444	****	
Site	0.0264	2	0.0132	0.127	0.881208	1	1	1.007473	****	
Tissue*Site	0.1154	2	0.0577	0.555	0.581075	2	1	3.062076		****
Error	2.4934	24	0.1039			2	2	3.124780	****	
						2	3	3.152786	****	

Table S2. Continuation.

Univariate Tests of Significance for log Ni (Metals.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable log Ni (Metals.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .07207, df = 54.000				
Effect	SS	Degr. of Freedom	MS	F	p	Tissue	Site	log Ni Mean	1	2
Intercept	2.408601	1	2.408601	33.41970	0.000000	1	3	-0.394840	****	
Tissue	1.550473	1	1.550473	21.51305	0.000023	1	2	-0.358168	****	
Site	0.026223	2	0.013111	0.18192	0.834174	1	1	-0.330322	****	
Tissue*Site	0.134333	2	0.067166	0.93194	0.400028	2	1	-0.117645	****	****
Error	3.891850	54	0.072071			2	2	-0.049710	****	****
						2	3	0.048537		****

Univariate Tests of Significance for Pb (Metals.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable Pb (Metals.sta) Homogenous Groups, alpha = .05000 Error: Between MS = 9.6499, df = 54.000				
Effect	SS	Degr. of Freedom	MS	F	p	Tissue	Site	Pb Mean	1	
Intercept	35219.38	1	35219.38	3649.697	0.000000	1	1	23.49000	****	
Tissue	0.43	1	0.43	0.044	0.834274	2	3	23.50000	****	
Site	13.55	2	6.78	0.702	0.499935	1	2	23.50000	****	
Tissue*Site	45.27	2	22.63	2.345	0.105497	2	2	23.67353	****	
Error	521.10	54	9.65			2	1	25.25714	****	
						1	3	25.94655	****	

Univariate Tests of Significance for log Zn (Metals.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable log Zn (Metals.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .17474, df = 54.000				
Effect	SS	Degr. of Freedom	MS	F	p	Tissue	Site	log Zn Mean	1	
Intercept	204.5309	1	204.5309	1170.497	0.000000	1	3	1.684224	****	
Tissue	0.8971	1	0.8971	5.134	0.027491	1	1	1.736241	****	
Site	0.1358	2	0.0679	0.389	0.679885	1	2	1.751616	****	
Tissue*Site	0.0964	2	0.0482	0.276	0.760040	2	2	1.907145	****	
Error	9.4359	54	0.1747			2	3	1.912528	****	
						2	1	2.086087	****	

Table S3. Comparison (Two-Way ANOVA followed by the Tukey HSD test) between mean values of biomarkers (ACAP, CAT, GST, LPO, and PSMT) in tissues (1: gill; 2: liver) of the euryhaline guppy *Poecilia vivipara* exposed (96 h) to three different sites (Sites 1-3) in a coastal water environment with long history of metal contamination.

Univariate Tests of Significance for log ACAP (Biomarkers.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable log ACAP (Biomarkers.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .01873, df = 28.000							
Effect	SS	Degr. of Freedom	MS	F	p		Tissue	Site	log ACAP Mean	1	2	3	4
Intercept	33.63201	1	33.63201	1795.390	0.000000		2	1	-1.61015	****			
Tissue	4.68638	1	4.68638	250.175	0.000000		2	3	-1.40381	****			
Site	0.13990	2	0.06995	3.734	0.036512		2	2	-1.13070			****	
Tissue*Site	1.62791	2	0.81396	43.452	0.000000		1	2	-0.85239		****		
Error	0.52451	28	0.01873				1	3	-0.77363		****		
							1	1	-0.26544				****

Univariate Tests of Significance for log CAT (Biomarkers.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable log CAT (Biomarkers.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .09587, df = 29.000							
Effect	SS	Degr. of Freedom	MS	F	p		Tissue	Site	log CAT Mean	1	2		
Intercept	86.73384	1	86.73384	904.7399	0.000000		1	2	0.786282	****			
Tissue	13.13646	1	13.13646	137.0294	0.000000		1	1	1.008088	****			
Site	0.00784	2	0.00392	0.0409	0.959994		1	3	1.096985	****			
Tissue*Site	0.55960	2	0.27980	2.9187	0.070004		2	3	2.037111		****		
Error	2.78012	29	0.09587				2	1	2.189639		****		
							2	2	2.348943		****		

Univariate Tests of Significance for log GST (Biomarkers.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable log GST (Biomarkers.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .02162, df = 22.000							
Effect	SS	Degr. of Freedom	MS	F	p		Tissue	Site	log GST Mean	1	2	3	
Intercept	2.436408	1	2.436408	112.6769	0.000000		1	2	-1.01486			****	
Tissue	6.065157	1	6.065157	280.4960	0.000000		1	1	-0.69911		****		
Site	0.330090	2	0.165045	7.6329	0.003036		1	3	-0.59452		****		
Tissue*Site	0.141508	2	0.070754	3.2722	0.057007		2	2	0.12699	****			
Error	0.475705	22	0.021623				2	1	0.16074	****			
							2	3	0.22969	****			

Univariate Tests of Significance for log LPO (Biomarkers.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable log LPO (Biomarkers.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .08479, df = 25.000							
Effect	SS	Degr. of Freedom	MS	F	p		Tissue	Site	log LPO Mean	1			
Intercept	17.16792	1	17.16792	202.4835	0.000000		1	3	0.501273	****			
Tissue	0.17351	1	0.17351	2.0464	0.164946		1	2	0.730768	****			
Site	0.25531	2	0.12765	1.5056	0.241328		2	3	0.784336	****			
Tissue*Site	0.07469	2	0.03734	0.4404	0.648658		1	1	0.837409	****			
Error	2.11967	25	0.08479				2	2	0.870888	****			
							2	1	0.876822	****			

Univariate Tests of Significance for MTLP (Biomarkers.sta) Sigma-restricted parameterization Effective hypothesis decomposition						Tukey HSD test; variable MTLP (Biomarkers.sta) Homogenous Groups, alpha = .05000 Error: Between MS = .01321, df = 30.000							
Effect	SS	Degr. of Freedom	MS	F	p		Tissue	Site	MTLP Mean	1	2		
Intercept	89.50003	1	89.50003	6777.346	0.000000		1	1	1.357661		****		
Tissue	0.00234	1	0.00234	0.177	0.676725		2	1	1.382351		****		
Site	0.80478	2	0.40239	30.471	0.000000		1	2	1.601266	****			
Tissue*Site	0.02885	2	0.01443	1.092	0.348371		2	2	1.682061	****			
Error	0.39617	30	0.01321				2	3	1.690003	****			
							1	3	1.747104	****			