Hatchability and survival of *Lamproglena clariae* exposed to increasing concentrations of aqueous aluminum

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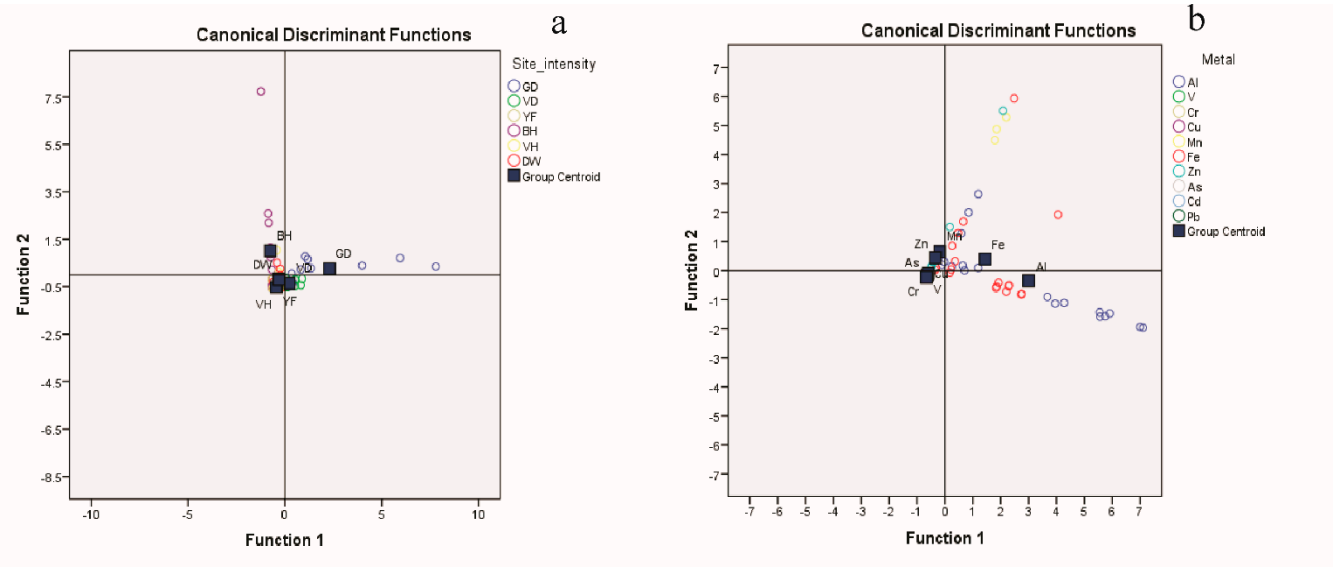
ALUMINUM AS EXPOSURE METAL

Water quality data from six different locations along the Vaal River (Grootdraai Dam (GD) (− 26.730831, 27.63125), Vaal Dam (VD) (− 26.894701, 28.145303), Yellowfish Paradise (YP) (− 26.730831, 27.63125), Bloemhof Dam (BD) (− 27.693728, 25.671511), Vaal-Harts Weir (VW) (− 28.114839, 24.925998) from a previous study (Pretorius and Avenant-Oldewage, 2021) was used to determine what metal should be used in a controlled study.

Sites compared to each other by using a Canonical Discriminant function grouped out according to metal concentration recorded in water and sediment samples at the six sampling sites as shown in Supplement Figure S1(a). The sites with overall higher metal concentrations recorded grouped together in the upper right quadrant of the graph with YP, VW, and DW. These sites were found to be the most "polluted" sites according to water and sediment metal analysis. Variables were pooled within-groups correlations between discriminating functions, Wilk's lambda p- value was found to be significant at test functions 1 through 5 with $p<0.05$. Fisher's linear discriminant functions for metal concentrations at sites (Supplement Table S1) indicated lower constant values at the identified polluted sites grouping YF=-383.3, VH=-487.7 and DW=-331.1 together.

Focusing on the influence the various metals might have on each other in Supplement Figure 1 (b) aluminum (Al) and iron (Fe) concentrations found at the six sampling sites grouped out from the other elements toward the right quadrants of the graph. Wilk's lambda p- value was found to be significant at test functions 1(water) and 2 (sediment) with $p=0.00$ and $p=0.12$. Fisher's linear discriminant functions for metal concentrations at sites (Supplement Table S2) indicate lower constant values at the identified polluted sites grouping Fe=-4.7 and Al=-9.1 out from the other

metals. Aluminum was chosen as exposure element in a controlled laboratory experiment due to its possible toxicity in aquatic environments.



Supplement Figure S1. Canonical discriminant functions between sites (a) and between elements (b).

Supplement Table S1. Fisher's linear discriminant functions for metal concentrations at sites.

Classification Function Coefficients

	Metal concentration per site					
	GD	VD	YF	BH	VW	DW
Al	851.0	798.5	332.6	672.5	443.2	313.5
V	3281.3	2603.9	1250.7	2345.6	1593.3	1112.1
Cr	-593.4	-395.0	-437.5	-263.1	-371.7	-405.1
Cu	-1719.6	-1567.9	-508.9	-1395.0	-810.0	-462.5
Mn	-312.5	-274.1	-111.2	-243.5	-155.5	-102.1
Fe	-10.4	-0.5	-2.1	-2.8	-4.1	-2.6
Zn	168.9	165.1	53.1	145.2	93.0	58.8
As	-2231.4	-1577.3	-896.8	-1404.7	-1045.4	-803.6
Cd	-388.7	-389.7	-124.2	-326.7	-207.8	-132.3
Pb	-221.5	-160.1	-81.4	-148.9	-103.5	-70.4
(Constant)	-1631.0	-1217.2	-383.3	-886.5	-487.7	-331.1

Fisher's linear discriminant functions

Supplement Table S2. Fisher's linear discriminant functions according to metal concentration in water and sediment.

Classification Function Coefficients

	Metal									
	Al	V	Cr	Cu	Mn	Fe	Zn	As	Cd	Pb
Concentration in water	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Concentration in sediment	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
(Constant)	-9.1	-2.3	-2.3	-2.3	-2.8	-4.7	-2.6	-2.3	-2.3	-2.3

Fisher's linear discriminant functions