Effect of lipopolysaccharides from *Microcystis* and *Lyngbya* on metal toxicity in *Fundulus heteroclitus*.

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Lipopolysaccharides (LPS's) are components of the cell walls of all Gram-negative bacteria and related Cyanobacteria. Often referred to as bacterial "endotoxin" or pyrogens (i.e. fever inducers), they have been recognized as the causative agent of sepsis and "toxic shock" associated with bacterial infection, and have more recently received attention related to the environment in association with 'harmful algal blooms'. Recently, Best, et al.[1] described the effect of cyanobacterial LPS's on the inhibition of glutathione S-transferase (GST) activity in zebrafish (*Danio rerio*) embryos. GST is an important detoxifying enzyme that catalyzes the conjugation of reduced glutathione to many potentially toxic compounds. Additionally, GST activity is normally increased after metal insult, and has been indicated as a first line of defense against Cd^{2+} toxicity before upregulation of metallothionein synthesis occurs[2]. We investigated the combined effect of cyanobacterial LPS extracts and $CdCl_2$ exposure in the killifish, *Fundulus heteroclitus*, to delineate a possible role of cyanobacterial blooms in the potentiation of metal toxicity.

Wild caught killifish from Northeast Creek, Mount Desert Island, ME. were transferred to 10 liter, high-density polyethylene tanks with running seawater at MDIBL where they were 1) exposed to LPS preparations from *Microcystis aeruginosa* or *Lyngbya* diluted into the test water and sampled throughout a time course for determination of GST activity (table 1.), and 2) exposed to several waterborne concentrations of $CdCl_2$, with and without addition of LPS's from either *Microcystis* or *Lyngbya*, to determine nominal LC_{50} values for Cd and examine the effects, if any, of LPS exposure on metal toxicity. For these studies, lyophilized LPS's from Cyanobacterial isolates were prepared by the method of Raziuddin et al.[3], using the "hot phenol/water" extraction method, and added to the appropriate experimental tanks at a concentration of 500ng/L. Cadmium, as $CdCl_2$, was prepared in 10mg/L concentration increments from 10-50mg/L for LC_{50} determination as shown in table 2. Additionally, samples were taken from 96 hr LC_{50} test organisms upon completion to measure hepatic glutathione S-transferase activity as summarized in table 3 below. The glutathione S-transferase kit from Cayman Chemical was used for analysis of samples as per manufacturer's directions.

Table 1. A time course study was conducted to evaluate the ability of lipopolysaccharides from the cyanobacteria *Microcystis* and *Lyngbya* to alter glutathione S-transferase activity in exposed killifish. Values are reported as the mean ±standard deviation of nmol conjugated CDNB/minute/mg protein according to the method of Habig et al.[4]. Time= 0 hrs is immediately before commencement of test. The '*Microcystis*" treatment had one additional time point of 32 hrs = 330.2 ±5.4. For all data n=3.

	0 hrs	0.5 hrs	1 hr.	2 hrs	4 hrs	8 hrs	16 hrs	24 hrs
Control	418.4 ±22.5	N/A	N/A	N/A	N/A	408.2 ±36.8	N/A	397.7 ±19.2
0.5ug/L *Microcystis*	428.2 ±36.5	254.3 ±42.2	302.8 ±31.7	311.6 ±28.3	299.4 ±16.2	318.9 ±31.1	332.6 ±25.7	348.9 ±2.1
0.5ug/L *Lyngbya*	392.2 ±26.7	288.2 ±38.6	271.4 ±13.8	298.6 ±40.1	310.3 ±6.2	309.4 ±67.8	341.2 ±60.2	382.6 ±40.9

Table 2. 96 hour LC_{50} values for killifish exposed to $CdCl_2$ in static seawater with semi-daily renewal. Values are reported as the mean ±standard deviation of probit analysis outcomes. An asterisk (*) indicates significant difference from the LC_{50} for $CdCl_2$ alone. For all measurements n=6, with 3 experimental repeats. Probit analysis was used for data determination.

LC_{50} for $CdCl_2$	43.091 ±4.45 mg/L
LC_{50} for $CdCl_2$ with the addition of 0.5ug/L *Microcystis* LPS	60.382 ±6.02 mg/L *
LC_{50} for $CdCl_2$ with the addition of 0.5ug/L *Lyngbya*	44.491 ±1.95 mg/L

Table 3. Glutathione S-transferase activity was measured in 1,000xg supernatants of Cd and Cd/LPS exposed killifish livers. Results are reported as nmol of conjugated CDNB/minute/mg protein. For all measurements n=3.

Treatment	0mg/L Cd	10mg/L Cd	20mg/L Cd	30mg/L Cd	40mg/L Cd	50mg/L Cd
$CdCl_2$	402.2 ±16.4	411.8 ±22.6	492.2 ±3.1	514.7 ±36.4	538.2 ±12.4	420.1 ±65.1
$CdCl_2$ / *Microcystis*	461.0 ±28.4	526.3 ±16.7	579.5 ±3.9	561.5 ±27.4	578.9 ±31.4	566.0 ±14.3
$CdCl_2$ / *Lyngbya*	382.1 ±40.9	404.6 ±24.3	484.5 ±28.9	530.8 ±43.6	527.3 ±36.3	508.1 ±29.2

As expected, when killifish were exposed to lipopolysaccharide preparations from either *Microcystis aeruginosa* or *Lyngbya sp.* a significant alteration in glutathione S-transferase activity was documented in the liver. This preliminary data shows a trend of an initial inhibition of GST activity by the cyanobacterial LPS's as early as 30 minutes, with a gradual recovery over a 24 hour period. Unexpectedly, instead of a potentiation of metal toxicity by cyanobacterial LPS, we recorded ameliorated toxicity values for $CdCl_2$ in toxicity tests that incorporated LPS preparations from *Microcystis aeruginosa.* Toxicity tests that incorporated *Lyngbya* LPS did not result in any significant change in LC_{50} value from that of tests with $CdCl_2$ as the sole variable. GST activity measurements taken from killifish after LC_{50} analysis imply that LPS from *Microcystis* enhanced GST activity in $CdCl_2$ combined exposures. However, these initial data need to be further examined to better understand interactions of a myriad of deleterious effects associated with both Cd and LPS toxicity.

These preliminary studies warrant further investigation regarding mechanisms of glutathione S-transferase interference by lipopolysaccharides, as well as exploration of possible processes involved in reduced sensitivity to metals in the presence *Microcystis* LPS. This work was funded in part by a New Investigator Award to G.D.M. from the Salisbury Cove Research Fund, Salisbury Cove, ME.

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