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

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Cyanobacteria are prevalent in the freshwater environment and can reach abundant mass in harmful algal blooms (HAB's). These blooms are common in aquatic environments with high nutrient influx and slow moving warm water, as found in the Florida Everglades[1]. 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 HAB's. The concurrent exposure of aquatic fauna to both LPS and metal is significant, as both are present in many aquatic environments, including the Florida Everglades. Recently, Best, et al.[2] 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, including metals. 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[3]. 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 containing static, Salisbury Cove seawater. Tanks were immersed in flow-through seawater to maintain constant temperature. Fish were 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 Cadmium and examine the effects of LPS exposure on metal toxicity. For these studies, lyophilized LPS's from cyanobacterial isolates were prepared by the method of Raziuddin et al.[4], using the "hot phenol/water" extraction method, and added to the appropriate experimental tanks at a concentration of 3.8 EU/L for *Microcystis* and 58.9 EU/L for *Lyngbya*. LPS concentrations were based upon *Limulus* amoebocyte lysate assay results. Cadmium, as $CdCl_2$, was prepared in 5mg/L concentration increments from 35-75 mg/L for LC_{50} determination as shown in Table 1. Concentrations of $CdCl_2$ used were based upon prior research and LC_{50}. Additional fish were also exposed to the same concentrations of Cadmium and LPS and samples of gill and liver taken at a variety of time points to measure glutathione S-transferase activity. A sample graph of hepatic glutathione S-transferase activity for *Fundulus* exposed to Cadmium and *Microcystis* or *Lyngbya* is shown in Figure 1. GST activity was measured using the glutathione S-transferase kit from Cayman chemicals and normalized to protein using the BCA protein assay kit from Pierce Biotechnology Inc. All kits were used to analyze sample per manufacturer's directions.

Table 1. 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. For all measurements n=6, with 3 experimental repeats. Probit analysis was used for data determination.

LC_{50} for $CdCl_2$	69.008 ± 1.029 mg/L
LC_{50} for $CdCl_2$ with the addition of 3.8EU/L *Microcystis* LPS	68.244 ± 9.021 mg/L
LC_{50} for $CdCl_2$ with the addition of 58.9EU/L *Lyngbya*	>75 mg/L

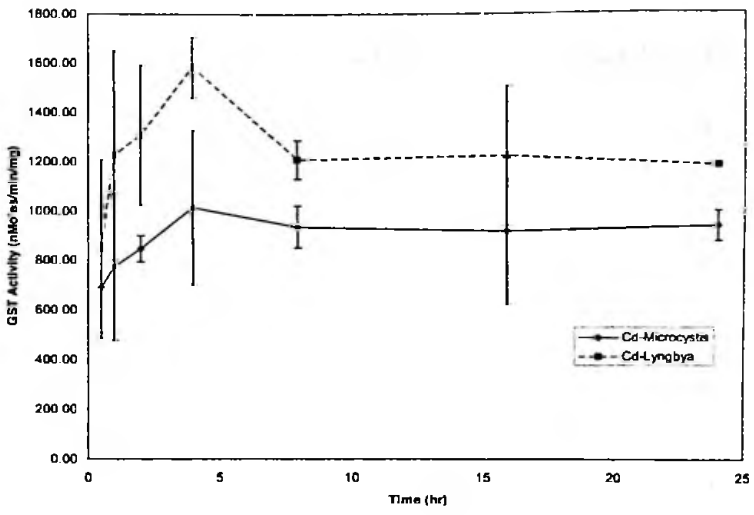

Fig. 1. This figure shows hepatic glutathione S-transferase in nMoles conjugated CDNB/minute/mg protein versus time in hours. *Fudulus heteroclitis* exposed to 55mg/L of $CdCl_2$ and either 3.8EU/L of *Microcystis* or 58.9EU/L of *Lyngbya*. Samples were taken at 0.5, 1, 2, 4, 8, 16 and 24 hours.

When killifish were exposed to lipopolysaccharide preparations from either *Microcystis aeruginosa* or *Lyngbya sp.* in addition to Cadmium, a change in glutathione S-transferase activity was documented in the liver. These data show a trend of higher hepatic GST in the *Fundulus* exposed to Cadmium and *Lyngbya* than in those exposed to Cadmium and *Microcystis*. Instead of the expected potentiation of metal toxicity by cyanobacterial LPS, we recorded ameliorated toxicity values for $CdCl_2$ in toxicity tests that incorporated *Lyngbya* LPS. Toxicity tests that incorporated *Microcystis* LPS did not result in any significant change in LC_{50} value from that of tests with $CdCl_2$ as the sole variable. These data, combined with the previous year's work at MDIBL[6] indicate that there may be a threshold level of LPS for the amelioration of metal toxicity, and at lower levels LPS may in fact potentiate metal toxicity. However, these data need to be further examined and additional experiments performed 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 potentiation and amelioration of metal toxicity by lipopolysaccharides, as well as exploration of GST and other detoxification processes involved in concurrent exposure with LPS and metals. 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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