Global Gene Expression in Larval Zebrafish Exposed to *Microcystis aeruginosa*: More Than Just Microcystin

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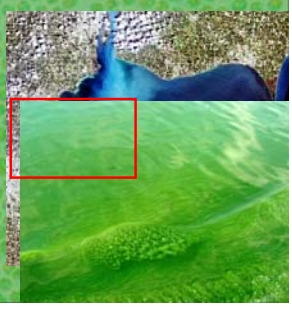
Toxic Algal Blooms

- Global Issue
- Examples
  - Saxitoxin, brevetoxin, *Pfiesteria*, *Lyngbya*, *Microcystis*
- Activities Affected
  - Human Health
  - Ecosystem Health
  - Commercial Fishing
  - Recreation & Tourism

Cost = $82 million/year

Microcystis Blooms

- *Microcystis ssp.*
  - Cyanobacteria
- Microcystin-LR
  - Toxin
    - 1µg/L- WHO advisory level
- Great Lakes
- Fish Kills?



Effects of Microcystin on Fish

- Mechanisms of Toxicity
  - Protein phosphatase inhibition
  - Hepatotoxicity
  - Possible ion regulation interference
    - Inactivation of Na+/K+ ATPase
- Current Research
  - Focus has been on acute exposures
  - Need more info on chronic environmentally relevant exposures
    - reproduction and development

Global Gene Expression

- Microarray
  - Measures expression of all genes in a sample.
  - Control vs. Treatment
    - Up-regulation
    - Down-regulation
- Benefits
  - Mechanism of Toxicity
  - Biomarker Development

Research Objectives

- Determine a suite of biomarker genes indicative of microcystin exposure in zebrafish.
- Monitor expression of these genes in later experiments designed to link changes in gene expression with higher level effects- chronic mortality, reproduction, histological lesions, etc.

Methods

- Zebrafish
  - Model organism
  - Sequenced genome
  - Commercially available arrays

Exposure

- Spawning
- Hatching:
  - 72-h post fertilization
- 50 larvae per beaker
- 3 replicates per treatment
- 96-h exposure
- Total RNA extraction

Treatments

- Purified Microcystin-LR (MC-LR)
  - 100 & 1,000 µg/L
- Vehicle Control
  - 0.05% ETOH
- Lyophilized *Microcystis aeruginosa* (LM)
  - Reconstituted in water to original concentration of 50 mg lyophilized cells/L (4.5 µg/L MC-LR)
- Negative Control
  - Zebrafish system water

Lyophilized *Microcystis* Microcystin-LR

Gene Expression Analysis & Statistics

- Affymetrix Core Facility, University of Tennessee
- Zebrafish arrays
  - ~15,000 probe IDs

- RNA Preparation
  - IVT/biotinylation
    - Message Amp™ II Biotin Enhanced Kit, Ambion
  - Chip hybridization, SAP staining, scanning
    - Affymetrix GeneChip Expression Analysis Technical Manual
- Partek® Genomics Suite™
  - Log base 2 transformation and GCRMA algorithm
  - ANOVA with FDR correction
    - 2-fold change
    - $p<0.05$

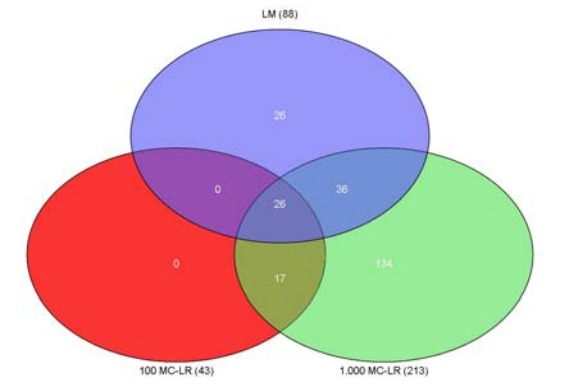
Results

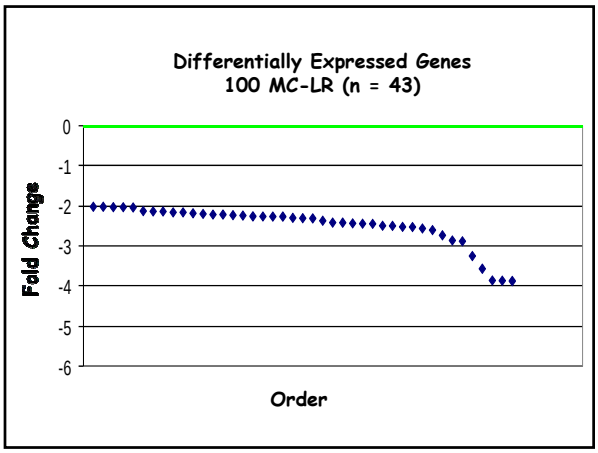
No significant mortality or developmental abnormalities

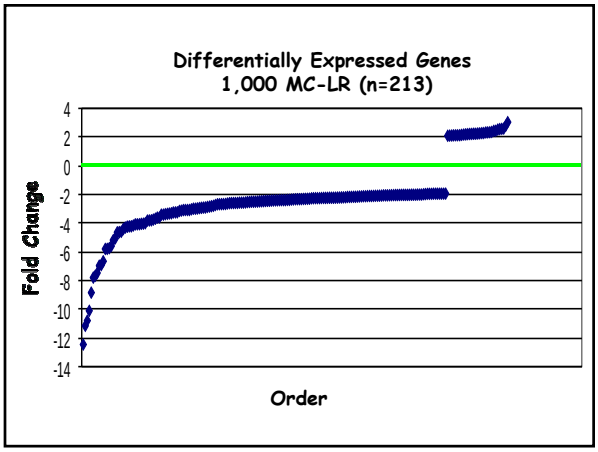
Number of Genes Up/Down-Regulated

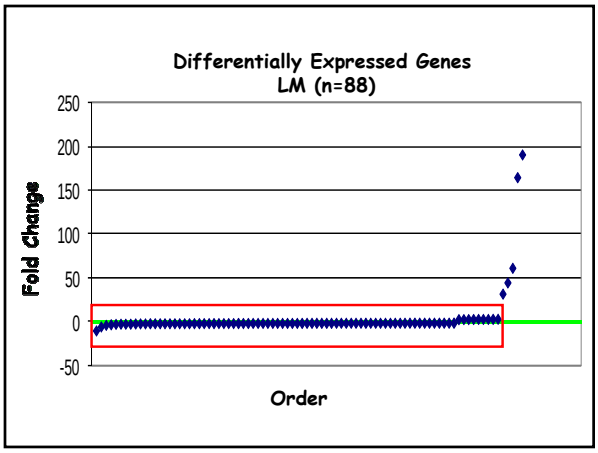
- 100 µg/L MC-LR: 43
- 1,000 µg/L MC-LR: 213
- Lyophilized *Microcystis*: 88

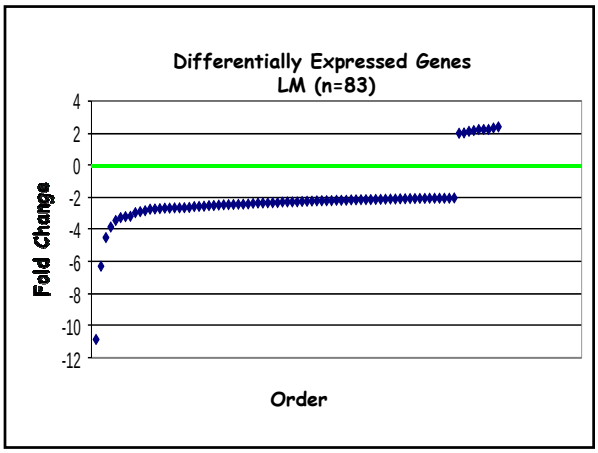
(2-fold change, $p < 0.05$)

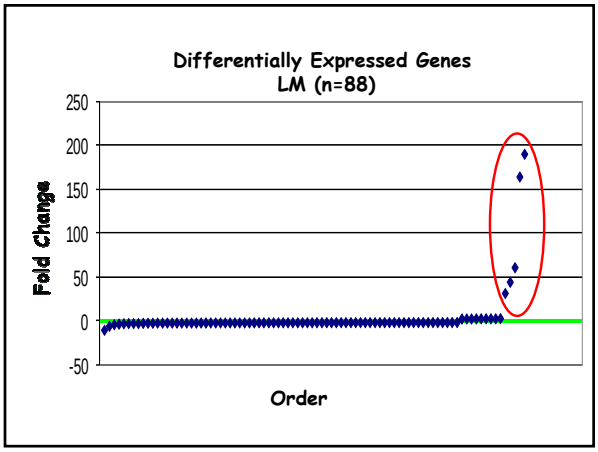



Differentially Expressed Genes
100 MC-LR (n = 43)
0
-1
-2
-3
-4
-5
-6
Fold Change
Order


Differentially Expressed Genes
1,000 MC-LR (n=213)
4
2
0
-2
-4
-6
-8
-10
-12
-14
Fold Change
Order


Differentially Expressed Genes
LM (n=88)
250
200
150
100
50
0
-50
Fold Change
Order





Up-Regulation of Vitellogenin Genes in LM Treatment

Gene Title	Fold Change	p-value
Vtg 3	31.2	<0.0000
Vtg 4	44.1	<0.0000
Vtg 2	60.8	<0.0000
Vtg 1,4,6,7	164.5	<0.0000
Vtg 1	190.6	<0.0000

Discussion

Vitellogenin

- Egg yolk precursor protein produced in liver in response to estrogens
- Should only be transcribed in reproductive female fish
- Highly up-regulated in lyophilized *Microcystis* treatment, but not in purified MC-LR treatment
  - Not a toxin effect

Implications

- *Microcystis* may produce a metabolite that is an estrogen mimic.
  - Not previously described in cyanobacteria?
  - Disruption of endocrine function in fish living in affected areas?
    - Reproductive impairment
    - Feminization of male fish

Summary

- Exposure to purified MC-LR and *Microcystis* resulted in differential gene expression in zebrafish larvae.
- Vitellogenin genes were highly up-regulated in *Microcystis* treatment, but not MC-LR.
- Additional data analysis will identify genes differentially expressed in both MC-LR and lyophilized *Microcystis* treatments, and these genes will be used as biomarkers in future experiments.

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