Assessment of Benthic Macroinvertebrate Community Impairment from High-Aluminum Acid Mine Drainage in Middleton Run, Ohio, USA and the Impact of Ingested Aluminum on Crayfish Growth

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- Metals in acid mine drainage can remain aqueous at low pH, but can precipitate at neutral pH

Differing Studies

- Studies differ on what impairs stream biodiversity the most
- Acidity vs Metals and Aqueous vs Precipitates

- Aquatic organisms found in streams impacted by acid mine drainage are far more sensitive to low pH and high acidity (Carlisle and Clements 1999)
- Acidity vs Metals, Aqueous vs Solids > gill filaments (Booth *et al.*, 1988; Neville & Campbell, 1988)
- Aqueous in lower pH, which is actually harmful

Study site: Middleton Run

- Tributary of Little Raccoon Creek
- Drainage covers 2.28 square miles
- AMD impaired
- Discharges nearly 130 pounds of Al/day into Little Raccoon Creek
- Reclamation of sites in 2005
- More reclamation in construction
- Never sampled for biological quality


Raccoon Creek
Little Raccoon Creek


Little Raccoon Creek Subwatershed
Middleton Run

Field Study

- Identify the benthic macroinvertebrate and fish assemblages found at three sample stations on Middleton Run.
- Analyze historical chemistry data at three sample stations on Middleton Run
- Examine relationships between historical chemistry data and biological data collected

- This objective tested the hypothesis that Middleton Run has poor biological diversity due to high aluminum concentration from acid mine drainage

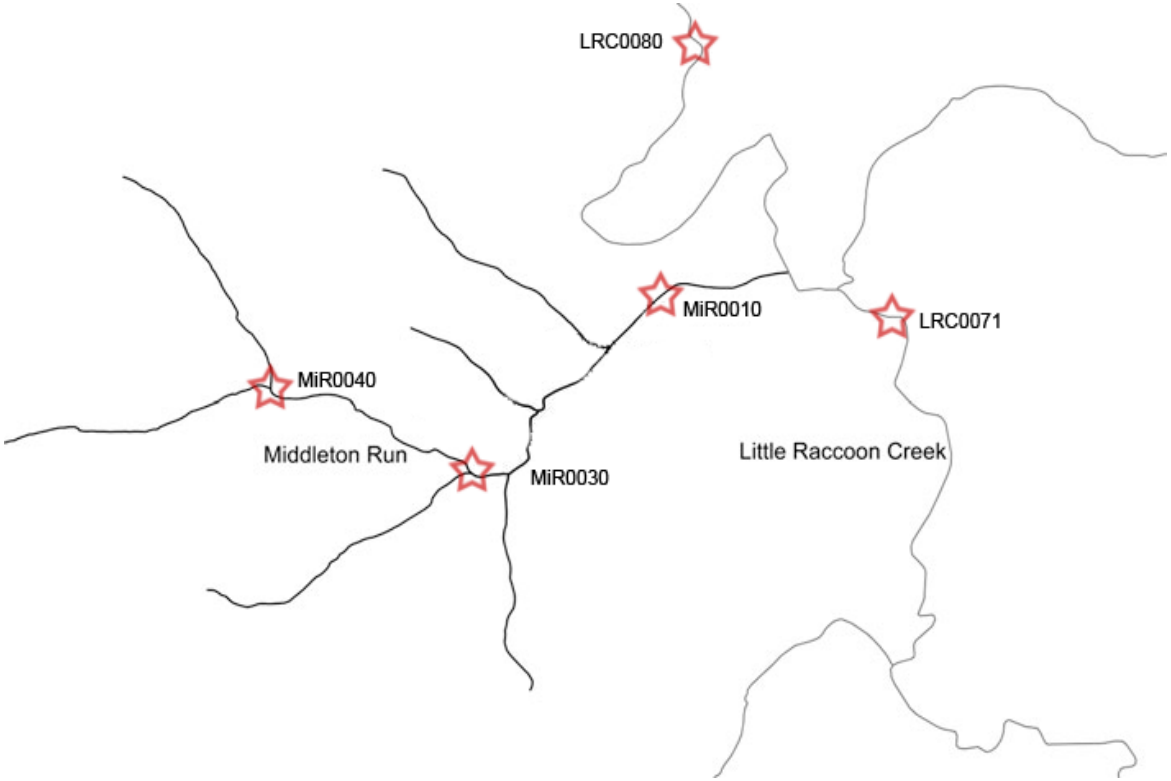
Laboratory Study

- Investigate whether aluminum-rich sediment impacts crayfish survival by reducing food quality
- Examined the impairment aluminum chloride spiked food pellets have on crayfish growth

- Tested the hypothesis that crayfish consuming higher amounts of aluminum would see a reduction in growth

Field Study Methods

- Stations were selected due to their proximity to key confluences in the sub-watershed



Field Study Methods

- Benthic macroinvertebrates collected using MAIS (*Macroinvertebrate Aggregated Index for Streams*) field sampling methods (Johnson 2006)
- Samples were placed in sample jars containing 70% ethanol & identified to the family level at a later time
- Fish samples via fish seines & long-line electroshocking in compliance with Ohio DNR and OEPA standards (OEPA 1988)
- Fish released after identification

Field Study Methods

- Examined historical stream chemistry data (watersheddata.com)
- MAIS score for each site was calculated from the identified macroinvertebrates – 9 bio metrics
- MAIS scores for each site were compared to historical stream chemistry data
  - Spearmans Rank Test correlations were run
- IBI (*Index of Biotic Integrity)* scores were calculated – 12 biological metrics

Laboratory Study Methods

- 60 wild crayfish were captured using dip nets, kick nets, and hands
- Obtained from unimpaired streams
  - Not previously exposed to high aluminum concentrations
  - Dairy Lane Creek & McDougal Creek
- Initially housed in large plastic bins for easy transportation

Laboratory Study Methods – Set up

- Crayfish placed in small plastic tubs
- Tubs aerated with air pump
- Use of R/O water with the addition of 26.4 mg/L marine salt
- Small PVC tube and aquarium plants used for habitat
- All exposed to identical temp and light cycle


2A
3A
4A
4B

Laboratory Study Methods –food pellets

- Crayfish ingestion of Al follows a methodology similar to that of Woodburn et al. 2011
- Food pellet made mixing 10 g of dried fish meal, *Spirulina,* 10 g of gelatin crystals, and 50 ml of hot water
- Treatment added
- Dried in syringes and cut into small 1mm pellets
- Why gelatin? Binder for medications

Laboratory Study Methods – Food pellet

- Dried food pellets were made following Gonzalez and Allan (2007)
- Dietary cellulose used as binder
- Dietary cellulose combined with fish meal for protein, wheat starch for carbohydrates, cod oil for lipids, and *spirulina*
- Treatment added
- Syringes used to extrude lines of mixture
- Baked in oven and cut into 1mm pellets

Laboratory Study Methods

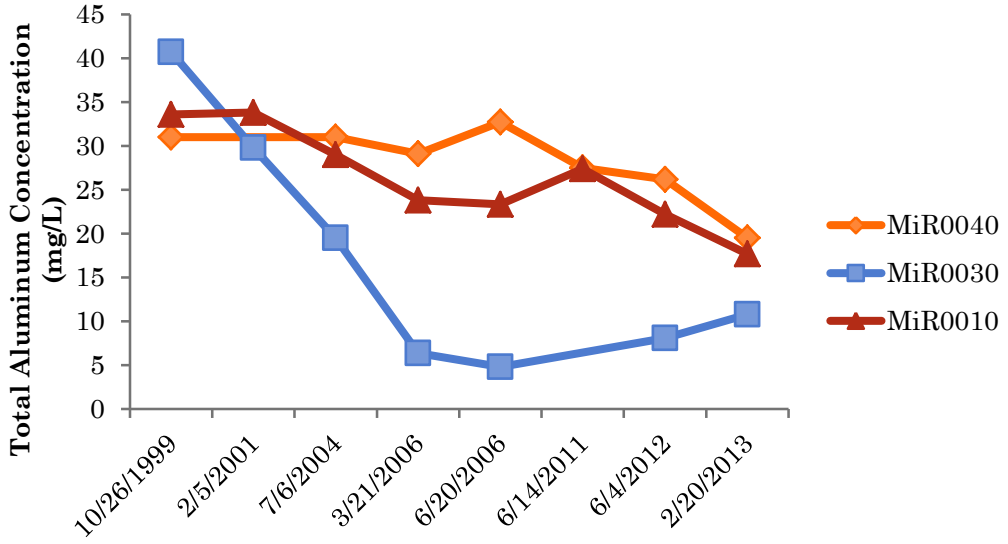
- Feeding Trial – Gelatin > Dry Pellets
- Some crayfish loss due to escape
- Four treatment groups of 12

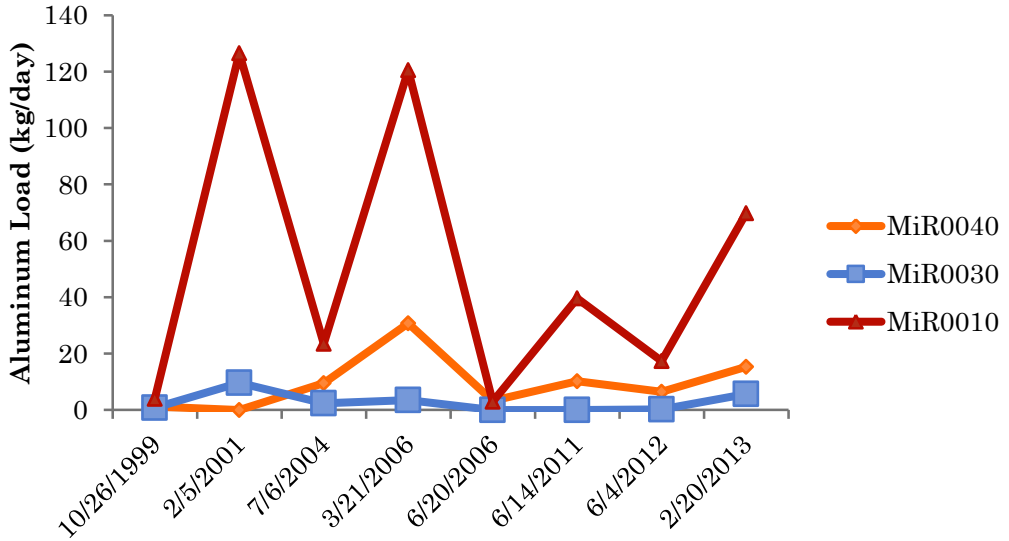
Laboratory Experimental Design

- Treatment groups fed different concentrations of aluminum chloride in spiked food pellets
- 50 mg/g treatment; 100 mg/g treatment,150 mg/g treatment; control group that received sodium chloride and no aluminum
- Fed one pellet each day for 6 weeks
- Crayfish carapace length, total length, and mass were measured prior to experimentation, at 2 weeks, 4 weeks, and 6 weeks

- Switch to dry pellets after one week
- Analyzed with Mixed ANOVA

Field Study Results





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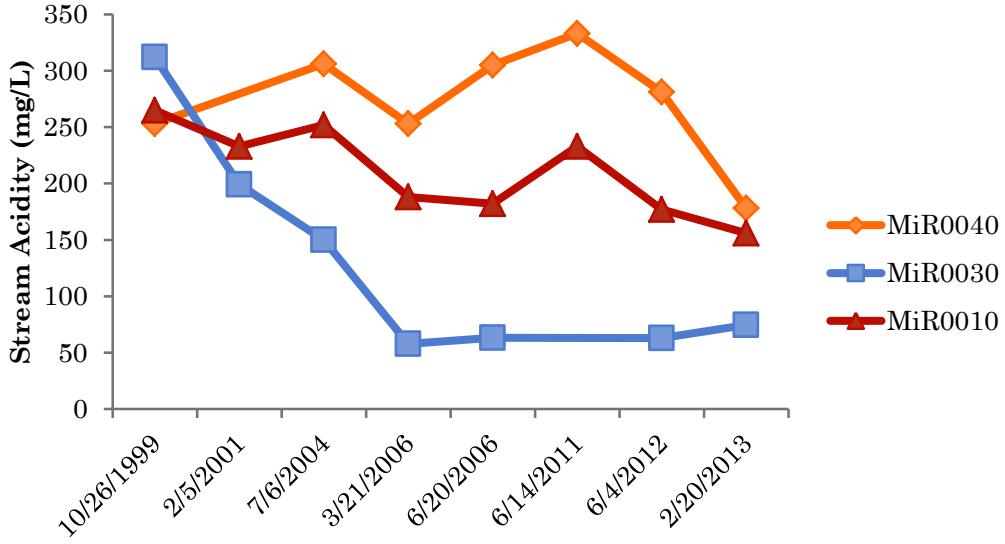

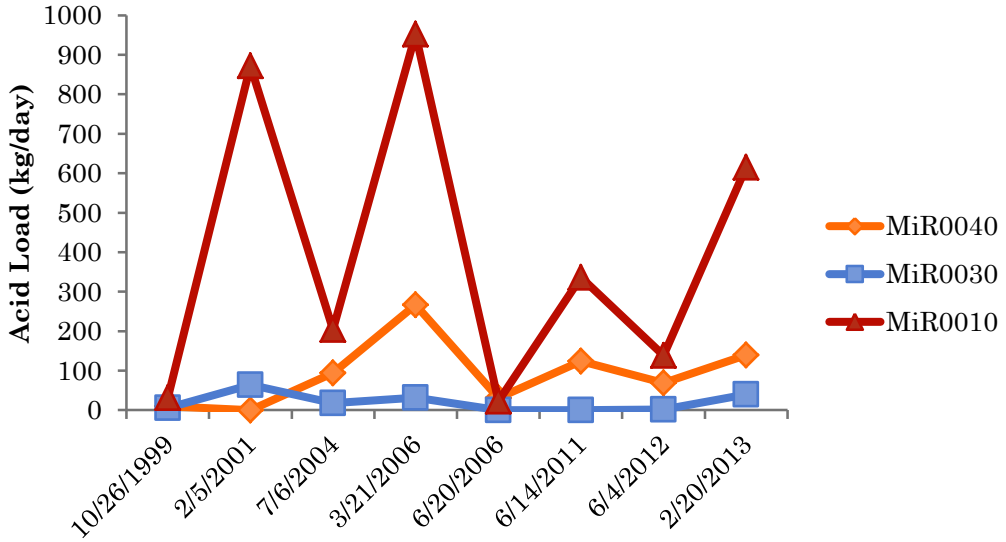

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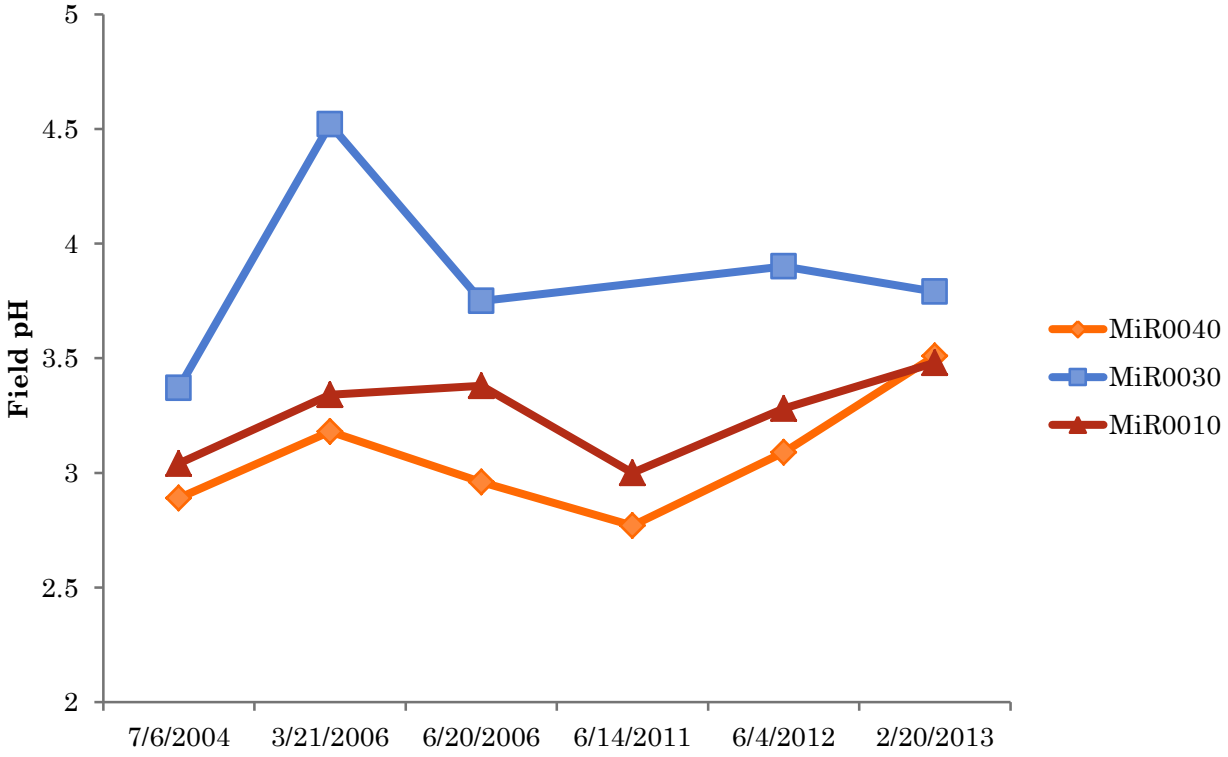
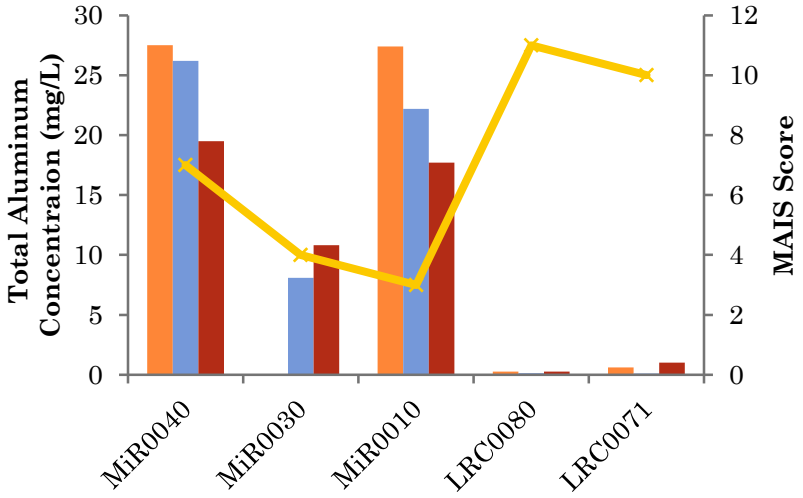
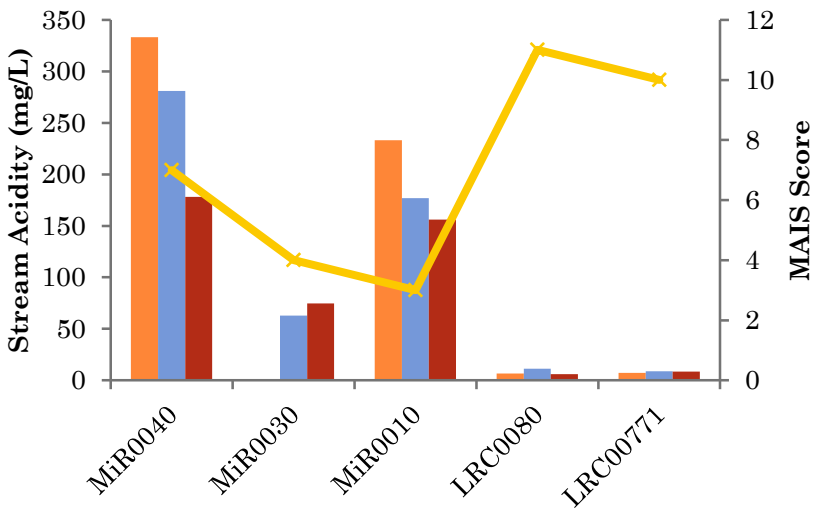
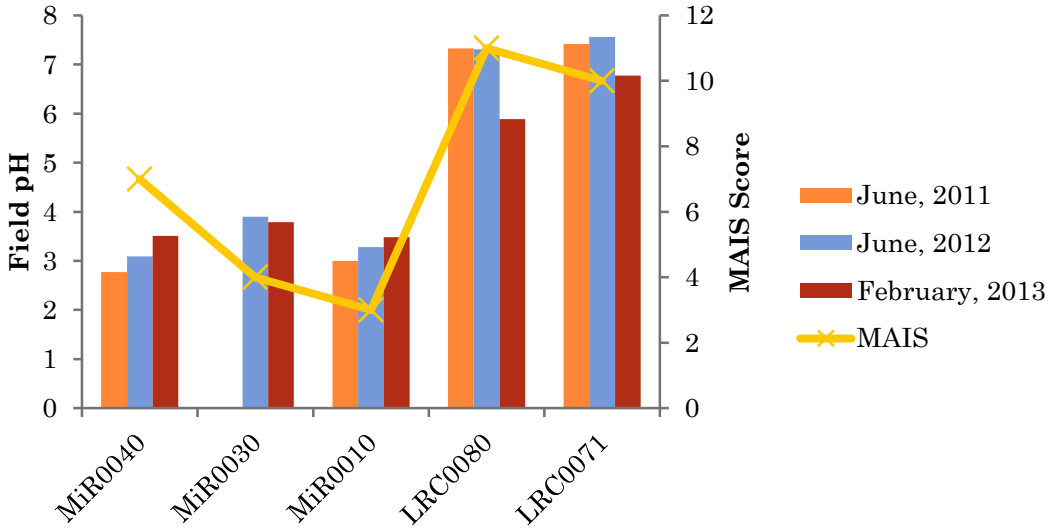


Site Number	MiR0040	MiR0030	MiR0010	LRC0080	LRC0071
MAIS Score	7	4	3	11	10
IBI Score	n/a	n/a	n/a	40	28

Field Study Results







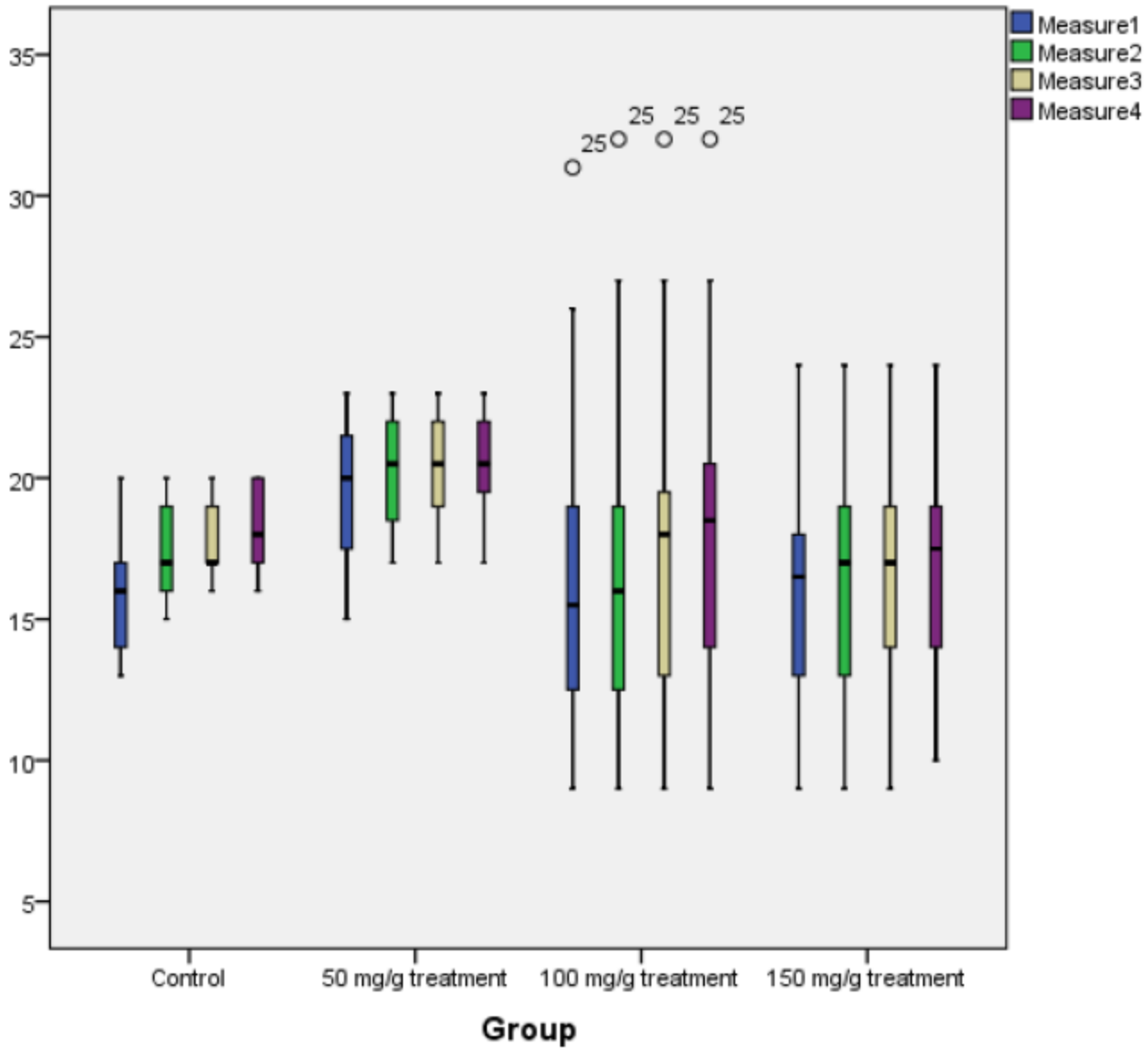
<u>Spearman Rank Correlation</u>

Aluminum = -.731

Acidity = -.843

Field pH = .718

Laboratory Results



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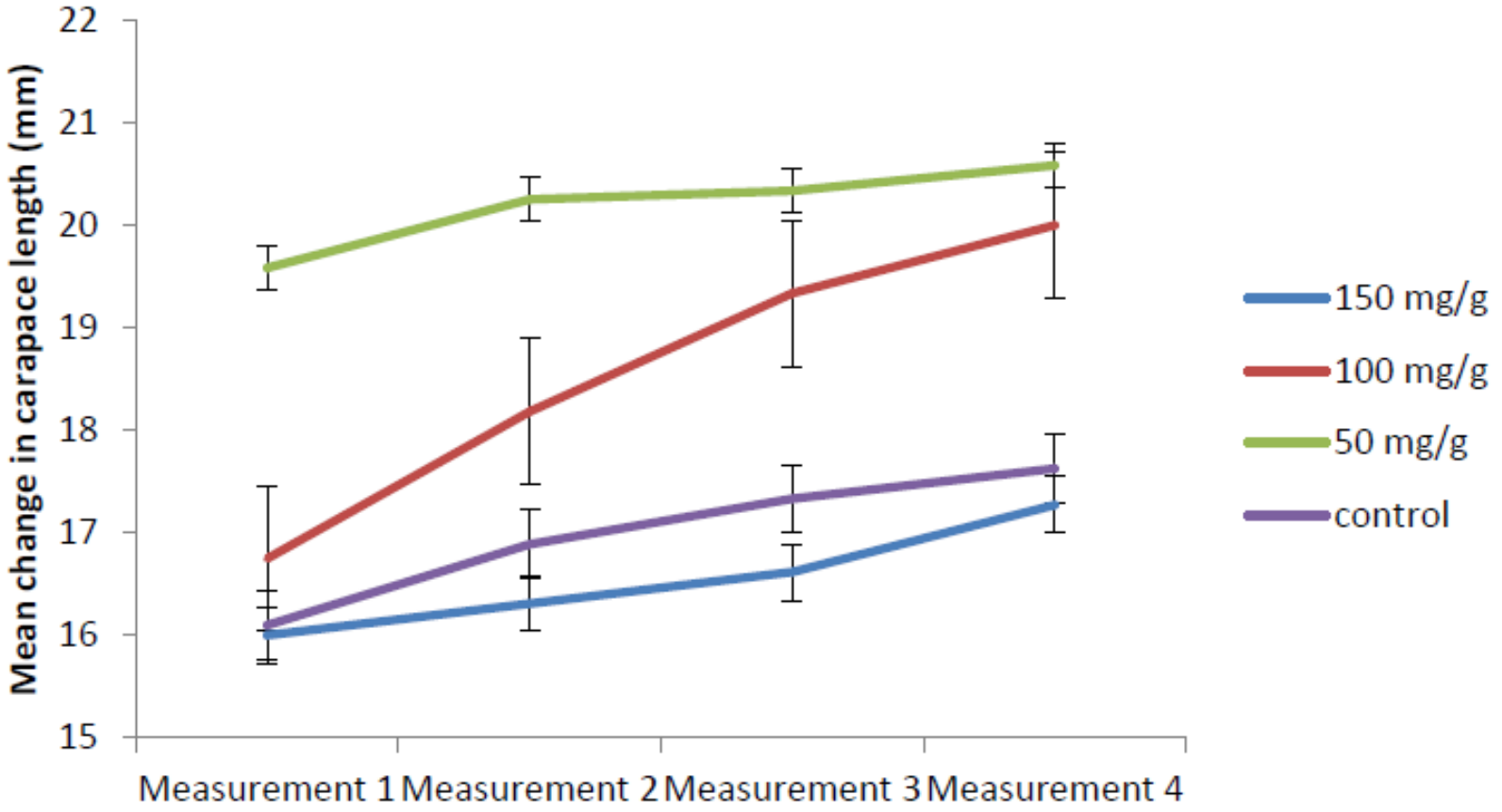

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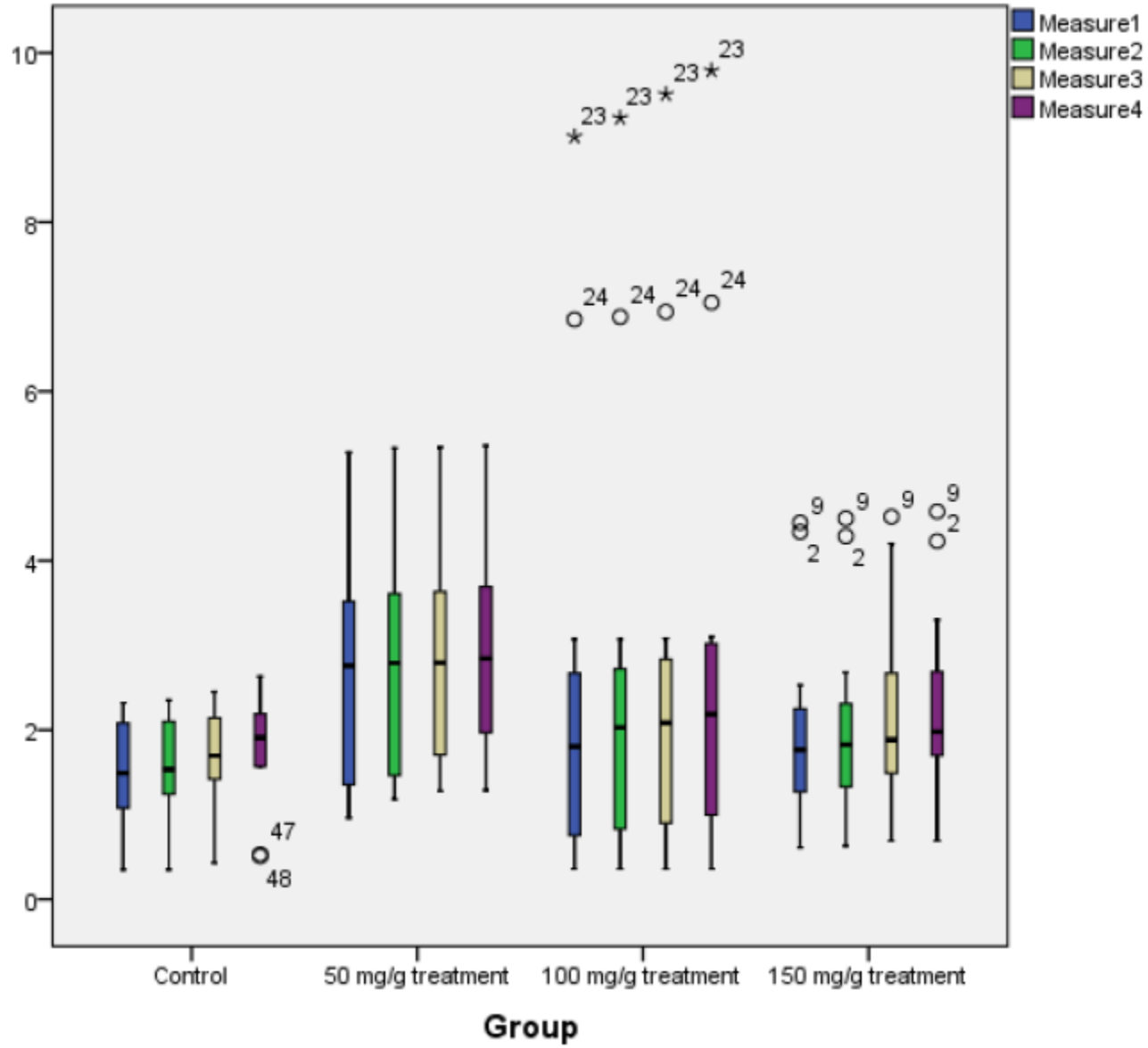

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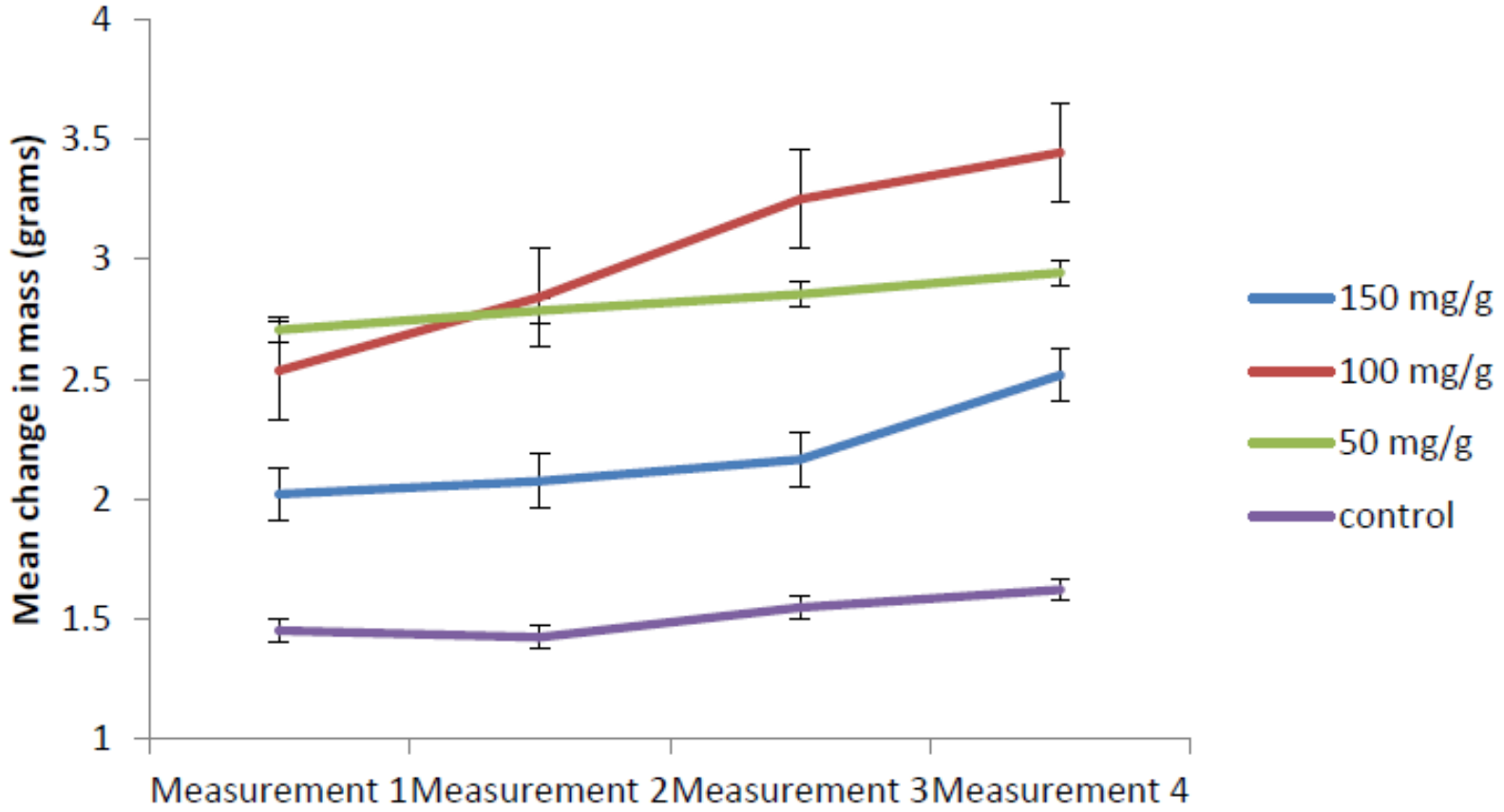


Field Findings

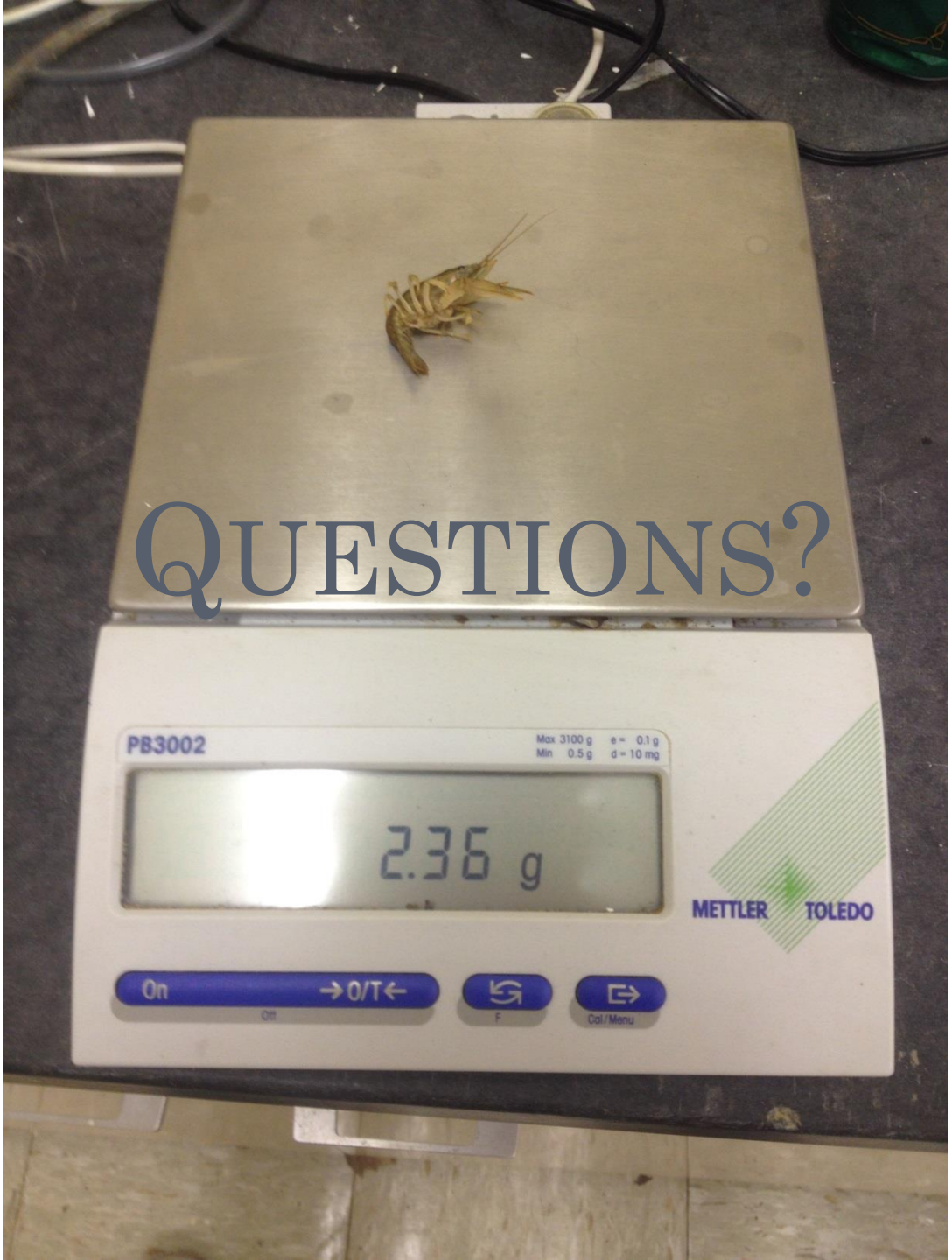
- The *very poor* quality of macroinvertebrate assemblages in Middleton Run was expected prior to sample collection
- It appears that impairment from metals like aluminum are likely a secondary impact with low stream pH acting as the primary reason for degradation of stream health
- MAIS scores in Little Raccoon Creek not altered by Middleton Run
- Poor IBI score found in Little Raccoon Creek may be due to loading from MiR0010

Lab Findings

- The crayfish growth results found following the 6 week feeding period were insignificant as growth was not impaired in any of the treatment groups
  - Supported by Clearwater et al. (2014)
- Possible that ingested aluminum is a metal that does not impair the growth of more tolerant macroinvertebrate species
- Also possible that the aluminum is excreted or bioaccumulated in fatty tissues, a finding that Woodburn et al. (2011) noticed

Future Outlooks

- If ingested aluminum has no impact on growth, impairment in streams must be due to another mechanism other than ingestion
- Future research can be done to examine other possibilities
- MAIS scores provide a baseline for future sample collection


PB3002
Max 3100 g
Min 0.5 g
e = 0.1 g
d = 10 mg
2.36 g
METTLER TOLEDO
On
→0/T←
Off
F
Cal/Menu

QUESTIONS?