

## Bioassays with the annelid *Lumbriculus variegatus*

Judith Roe<sup>1</sup>, Karoline Dillenbeck<sup>1</sup> and Erika Bernard<sup>2</sup>

University of Maine at Presque Isle<sup>1</sup> and Presque Isle High School<sup>2</sup>

The annelid *Lumbriculus variegatus* has been widely used to assess the effects of toxic pollutants. They are aquatic annelid worms that are easy to maintain in the lab and are available from Carolina Biological Supply. We have developed two assays for testing the effects of contaminants in water using these worms that could be performed in class with well water. Our primary reference is O’Gara et al. 2004.

O’Gara, B. A., Bohannon, V. K., Teague, M. W., & Smeaton, M. B. (2004). Copper-induced changes in locomotor behaviors and neuronal physiology of the freshwater oligochaete, *Lumbriculus variegatus*. *Aquatic toxicology*, 69(1), 51-66.

### Behavior Assay:

The worms display different escape reflexes upon being touched on either the head or the tail and so we developed a bioassay for the effects of metals on their behavior. We piloted this bioassay with copper sulfate solutions and one well water that had high Arsenic levels and saw an effect with each.

When you touch the head of the worm with a blunt object (we used plastic paddles), it displays the “body reversal” behavior. The worm jumps end to end and swaps its head and tail placement. See movie below and set playback speed at 0.25 in the Settings (if on Google Drive)



Body Reversal 1.mov

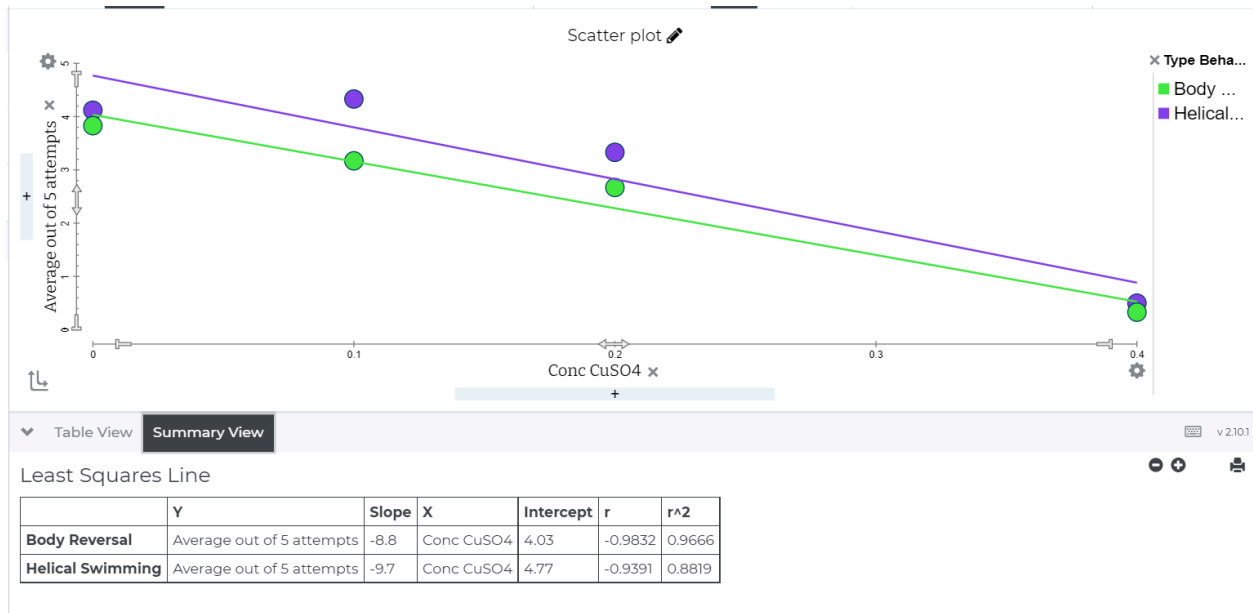
When you touch the tail of the worm with a blunt object, the worm displays a “helical swimming” behavior where the worm quickly swims off in a helical pattern. See movie below.



Helical Swimming 2.mov

We placed 6 *L. variegatus* worms into individual petri dishes with 30 mL of copper sulfate solutions in Poland Spring spring water and placed them in an incubator overnight at 21 degrees C in the dark. We tested 0, 0.1, 0.2, 0.4 and 0.8  $\mu\text{M}$  copper sulfate in Poland Spring Water and 8 ppb Arsenic in well water. After 18 hour we performed the bioassay. We touched each worm at either the head or the tail 5 times with 5 seconds in between and counted how many times the worm exhibited the escape behavior (head-body reversal; tail-helical swimming). Results were collected as # behaviors/5 and the averages of the 6 worms at each concentration are plotted below. As the concentration of copper sulfate increased, we saw a decrease in the average number of escape behaviors exhibited. The average number of escape behaviors seen in worms in the high arsenic well water was less than worms in control spring water.

[link needed to dataset for TUVA HERE]



### Regeneration Assay:

*L. variegatus* worms show a remarkable ability to regenerate a new head or new tail when the body is split in two. In fact, they perform asexual reproduction by splitting themselves in half, i.e. fission. The worms can grow a new head or new tail within one week. We tested the effect of copper sulfate and high arsenic on regeneration in the worms. Each worm was placed briefly on a wet filter paper circle and cut in two with a scalpel. The two halves were then placed in a petri dish with the filter paper and 30 ml of solution. Worms were assessed for regeneration after 1 week or 2 weeks. It might be a good idea to try the control worm first and become acquainted with how new heads and new tails look. A dissecting microscope is needed. Note: Photography is challenging. We will try nicotine anesthesia in the future.

One reference is:

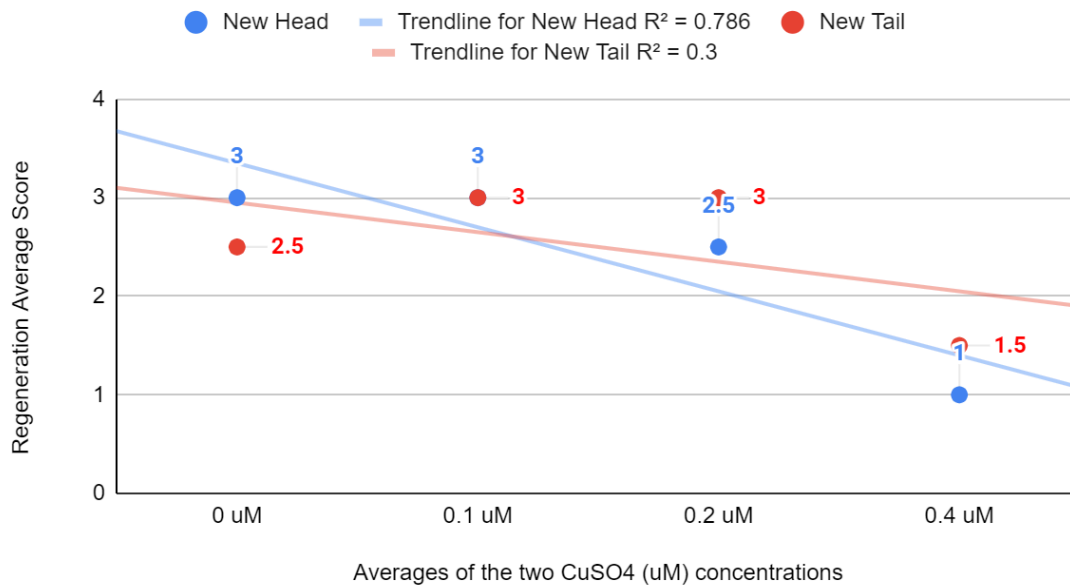
Martinez, V. G., Reddy, P. K., & Zoran, M. J. (2006). Asexual reproduction and segmental regeneration, but not morphallaxis, are inhibited by boric acid in *Lumbriculus variegatus* (Annelida: Clitellata: Lumbriculidae). In *Aquatic Oligochaete Biology IX* (pp. 73-86). Springer, Dordrecht.

A pilot experiment with 2 worms at each concentration of Copper Sulfate (CuSO<sub>4</sub>) after 1 week.

Well water with Arsenic with 8 ppb did show an effect in this assay (average was 1)

Regeneration Score	
0	These are dead and <b>will not be counted in the chart</b>
1	Little to no growth (or very small)
2	It got bigger than it was (small)
3	Just good or average
4	Long or lots of regeneration

## Regeneration of Head and Tail in CuSO<sub>4</sub> concentrations



These photos were taken after two weeks. We are still learning how to shoot these as they move fast.



Control- dH<sub>2</sub>O: New head is on the LEFT (new head on left is pinkish, old tail on right is bluish)



Control- dH<sub>2</sub>O: New tail is on the LEFT (old head on right is bluish-new tail on left is pink)



0.4  $\mu\text{M}$   $\text{CuSO}_4$ : Short new tail is on the LEFT



8 ppb Arsenic well water: Stubby new tail is on the LEFT