Assessing Blackworms as a Model for Studying Avian Vacuolar Myelinopathy

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Introduction

• Avian Vacuolar Myelinopathy (AVM) is a fatal neurological disease that affects numerous species of birds in the Southeastern United States.
• The disease has been linked to a species of cyanobacterium (Aerolothron hydridicala) that grows on hydrilla, an aquatic plant. It is believed that this cyanobacterium produces a toxin which causes the disease when eaten.
• Although this disease has severe effects on birds, studies have shown that some species of fish, turtles and invertebrates are also susceptible to the toxin.

• Lumbriicus variegatus (California blackworm) is a species of worm that inhabits shallow water environments such as marshes, swamps and ponds.
• They are often used as models in toxicity testing due to their low level of maintenance and cost efficiency.
• Blackworms reproduce asexually via fragmentation, and regeneration can be used as an endpoint in toxicity testing.

Methods

Exposure to Toxic Extracts

- Whole worms of approximately equal size were exposed to methanol extracts of hydrilla from AVM+ and AVM (control) locations
- Extracts were filtered prior to dosing using Amicon Ultra Centrifugal Filters, 3kDa
- Worms (2 per treatment) were placed in 24 well plates in 2 mL spring water, plus extract (20 µL, 10 µL, 5 µL per well for each extract)
- 5 day exposure; mortality was assessed daily

Exposure to Environmental Samples

- Water and sediment samples were collected from Cherokee Recreation Area at J. Strom Thurmond Lake, GA (an AVM location) on 11/11/2016, 12/2/2016, and 12/17/2016
- Control sediment was collected 01/14/2017 from Lake Hartwell, SC (an AVM location). Spring water was used as a water control.

Water Exposure Regeneration Tests

- Head and tail regeneration was measured in each lake water sample and control
- Head and tail segments of each worm (10 per treatment) were removed with a scalpel; tail and middle segments were counted
- Middle segments were placed in a 24 well plate with 2 mL of treatment water
- Regenerated segments were counted after 2 weeks and average number in each group was compared to controls via t-test. Worms are expected to grow back 8 head segments, regardless of the number removed. Tail segment regeneration is expected to match the number removed.

Sediment Exposure Reproduction Test

- Dried sediment (30 x 1 g) from each collection date was mixed with dried nettle powder (food source) and placed into a glass jar. 100 mL of spring water was added.
- 10 worms of approximately equal weight were added to each jar
- Water was constantly aerated over the course of the experiment and was partially exchanged with fresh spring water after 2 weeks
- After 28 days, all worms were collected and counted

Results (Continued)

• Water Exposure Regeneration (Continued)
- Two worms, one control and one Cherokee 12/17/16, grew abnormal head or tail segments (see Figure 3). Since only two such worms were observed, and one of them was in the control, this abnormality does not appear to be associated with the AVM toxin.

• Sediment Exposure Reproduction Test
- The control sediment had the highest reproduction rate. Lake Thurmond water samples from earlier dates were found to have more worms than later dates (see Figure 4).

Discussion

• Blackworms do not appear to be acutely sensitive to the AVM toxin, as no mortality was seen in the experiments with toxic extracts.
• The head regeneration test does not seem to be a sensitive endpoint. The data from the test show that the average of the head segment regeneration between all four water treatments ranged around the same number.
• There was a significant difference in the tail regeneration but due to limited number of replicates and a high mortality rate additional testing must be done.
• In the sediment tests, fewer worms were collected in the lake sediments verses control. However, this test needs to be repeated to gain more replicates and test additional control sediment.

Conclusions

• From the tests done so far it is still difficult to determine whether blackworms are a good model for testing AVM. There were no deaths of intact worms in the water environments with the toxin nor was there evidence to support that the toxin affects their regeneration.
• The regeneration and reproductive tests need to be repeated, and additional tests with different endpoints should be conducted.

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