Arsenic-Induced Gut Dysbiosis and Behavioral Activity in Chinese Mystery Snails: Exploring the Microbiome–Nervous System Link.

Alejandra Gonzalez, Ever Alves, Sarah Alaei, and Marc Nahmani

University of Washington Tacoma

TBIOMD 495

BACKGROUND

- Gut dysbiosis can serve as a biomarker for poor health outcomes across multiple species. The gut-nervous system signaling network in invertebrates can serve as a gateway to deepen our understanding of the gut microbiome's influence on the nervous system, analogous to the vertebrate gut-brain axis.
- Our research used Chinese Mystery Snails (CMS), an invasive species commonly found in freshwater systems in the Pacific Northwest, to examine how arsenic exposure disrupts the CMS gut microbiome and how it could affect the nervous system, evaluated through their feeding and mobility behaviors as well as the presence of arsenic-metabolizing bacterial genes in the snail gut.

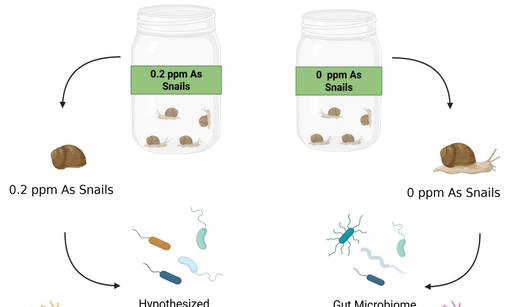


Figure 1. Hypothesized Model of As Impacts on CMS microbiome and Behavioral State. Snails were kept in jars with either 0.2 ppm or 0 ppm of As in the water. Physical alterations in the gut microbiome caused by arsenic lead to distinct gut microbiome DNA and compromised neurological function. Our experiments aimed to determine whether altered neuronal function, impacted by the microbiome, was due to As. We tracked the altered Neurological function via the behavioral state and the microbiome with altered DNA.

EXPERIMENTAL DESIGN

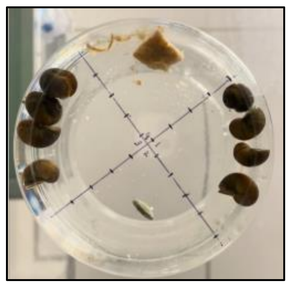


Figure 2: Behavioral Assay Setup. 500-ml beakers were divided into four quadrants, with snails alternating with food. Eight snails total. An algae wafer, and "snello" used as food.

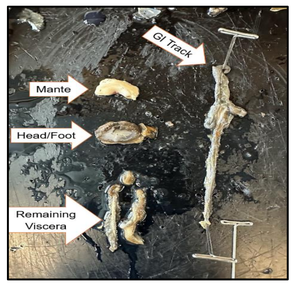


Figure 3: Snail Anatomy/Dissection. Image taken from Kovalchuk, Rockefeller, Wairimu, et al., manuscript in prep, showing the GI tract, mantle, head/foot, and remaining viscera.

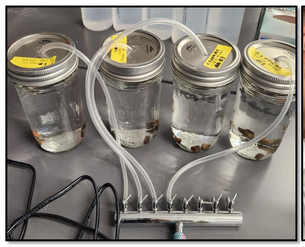


Figure 4: Experiment Setup. Eight snails were kept in six different jars with different [As](v). Two jars per treatment group. 0 ppm (parts per million), 0.2 ppm, and 2 ppm. The figure has only four jars due to the high mortality rate in the 2 ppm group.

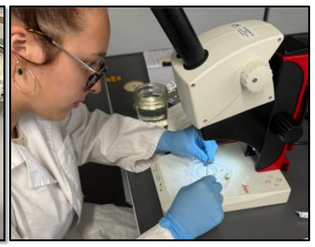
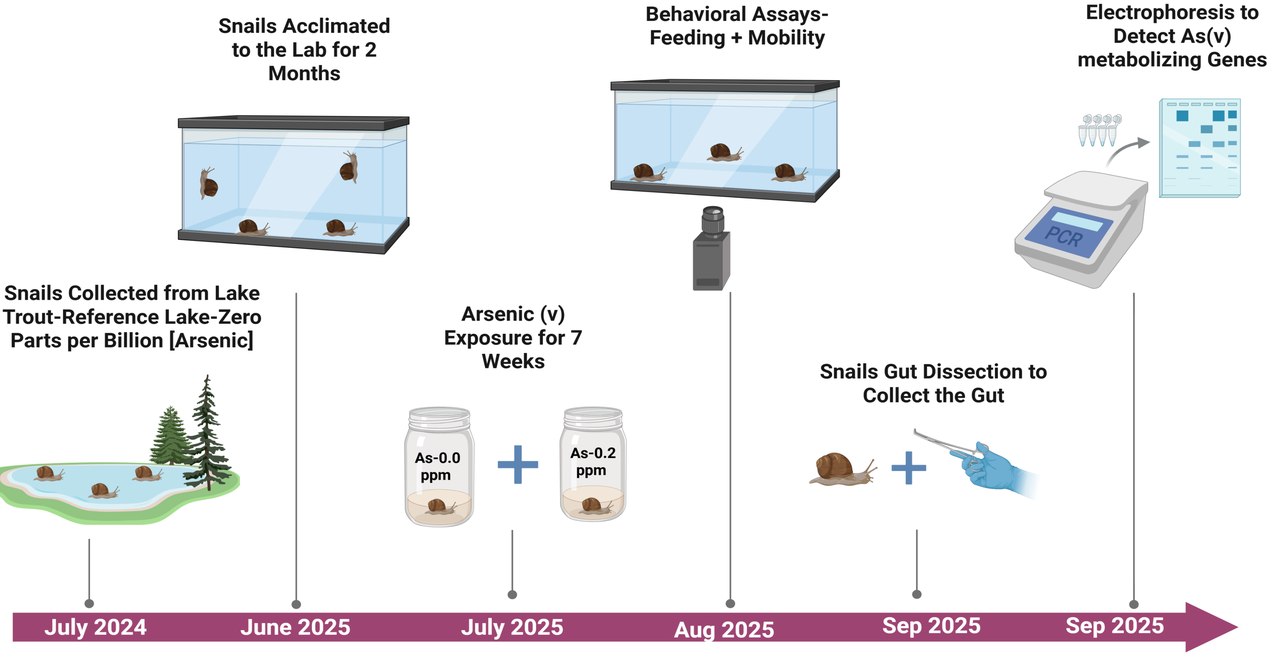


Figure 5: Snail's Gut Dissection. At the end of exposure, the snails were dissected to extract gut tissue for DNA analysis of gut microbiota composition using 16S rRNA gene sequencing via Oxford Nanopore.

METHODS



RESULTS

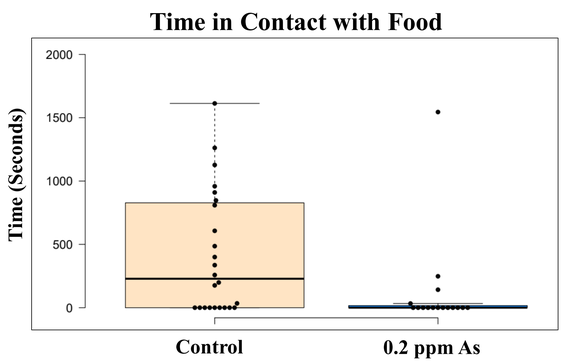


Figure 6. Reduction of Arsenic-treated CMS Time in Contact With Food. This graph shows time (seconds) the snails spend in contact with food. The black horizontal bars represent the mean time spent in contact with food. The individual black dots represent individual data points (snails). We used the Mean Whitney U test, two-tailed, with p-value = 0.02. 0.2 ppm snails exposed for seven weeks. Control N= 24 snails and 0.2 ppm [As] N= 16 snails.

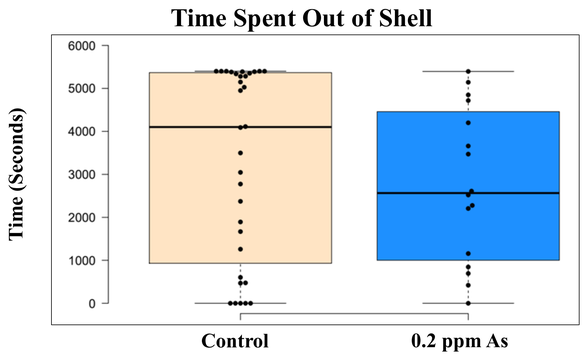


Figure 7: Comparison between Untreated and Arsenic-treated snails, and when out of the shell. This bar graph shows the average time in seconds that snails spent out of their shells. The black horizontal bars represent the mean in each group. The individual black dots represent individual data points (snails). Data shows a p-value of 0.25. We used the two-tailed Mean Whitney U test for the p-value calculation. 0.2 ppm snails exposed for seven weeks. Control N=33 snails and 0.2 ppm [As] N= 16 snails.

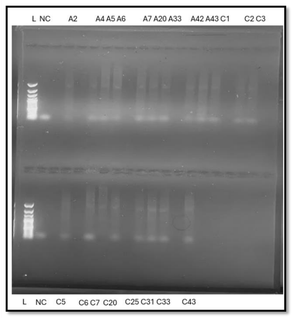


Figure 8a. arsM Gel.

Figure 8a. arsM Metabolic Gene Evaluation with the Snail Gut DNA. *arsM* Primer, standard ladder (L), Negative control (NC) possessed standard master mix and primers with water replacing DNA. Majority of the DNA had non-specific amplicon except for the occasional failed synthesis and faint contamination.

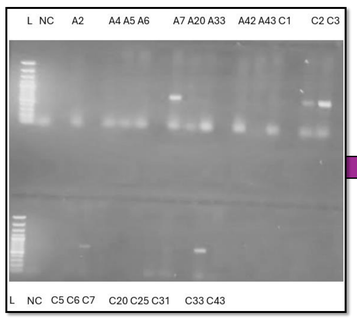


Figure 8b. arrB Gel.

Figure 8b. arrB Metabolic Gene Evaluation Tested with the Snail Gut DNA. *arrB* Primer, standard ladder (L), Negative control (NC) possessed standard master mic and primers with water replacing DNA. Most of the DNA failed to show any indication of band in a different location, however A7, C2, C3, C6, and C33 were successful in amplicon.

DISCUSSION

- For both variables, time out of the shell and time in contact with food, the data support our initial hypothesis that the arsenic-treated group would present lower activity. Only Time in contact with food shows statistical significance. However, no causation can be established due to high variability in the individual data points.
- The arsenic metabolic gene evaluation shows we had 1/9 of the As-treated samples positive for *arsB* and 3/11 of the controls positive for *arsB*, which does not suggest arsenic was metabolized more efficiently in the arsenic-exposed group.
- Further investigation, accounting for other variables such as sex, time of exposure, light conditions, and age, may yield significant insight into behavioral outcomes.

FUTURE WORK

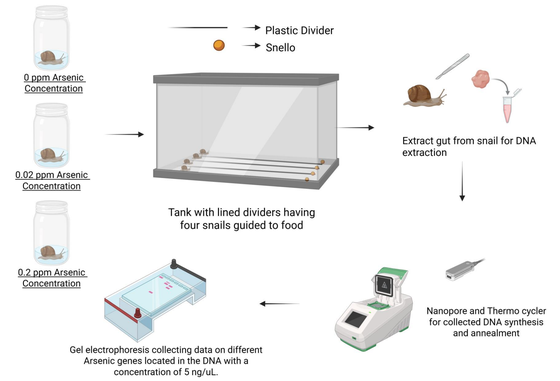


Figure 9. Future Directions: Using Adult CMS to assess the alterations in the behavioral state and microbiome. Revisions to the test would focus on using adult snails raised in different concentrations of ppm, with no more than 0.2 ppm arsenic. Snails are placed in a large tank lined with clear plastic walls to track the movement of the snails without interference. Snello would be placed at the end to motivate behavioral activity. Snails' guts will be collected after observations for several weeks, where immediate DNA lysis will occur, then synthesized and diluted for primers and mastermix to ID sources of prokaryotic DNA. The goal of this experiment is to try and eliminate the error of having more tissue than gut using a greater mass of snail via the adults. This would also allow for a greater amount of concentration when trying to synthesize the data, and hopefully lead to a more relevant connection between the microbiome aspect of this experiment.

ACKNOWLEDGEMENTS

Figure 2. Experimental Design Planning for Methodology. Designing the project spanned about four to five weeks, where learning was integrated with methodology to determine the best way to test for neuronal and microbiome correlation.

- We cannot express our immense gratitude to Dr. Alaei and Dr. Nahmani for sharing their expertise with us, helping us acquire valuable transferable research skills. We thank our fellow 14 students from the summer 2025 TBIOMD 495 class.