Arsenic Bioaccumulation and its Effect on the Microbiome and Behavior of Juvenile Chinese Mystery Snails

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Introduction

- Arsenic (As) is a legacy contaminant in south-central Puget Sound lake ecosystems due to emissions from the former ASARCO copper smelter (Fig. 1).
- Chinese Mystery Snails (CMS), a ubiquitous aquatic snail found in such lakes, have been identified as accumulating high levels of As due to their role as primary consumers.
- Little is known about the impacts of environmental contaminants such as As on invertebrates, specifically molluscs, and how biotransformation processes occurring within these lower trophic level organisms may shape the dynamics of As throughout the broader lake system.
- Our research aims to characterize the degree to which juvenile CMS bioaccumulate As over the course of an exposure period, if there are predictable shifts in microbiome structure and behavior, and if those shifts differ depending on the life stage in which exposure occurs.

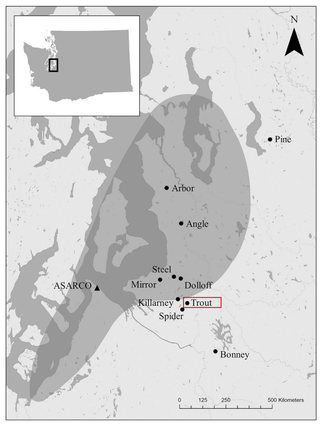


Figure 1. Map of the predicted ASARCO smelter plume range, which is represented by the shaded area. Smelter location and study lakes also indicated, with Trout Lake outlined in red (Adapted from Hull et al. 2023).

Results

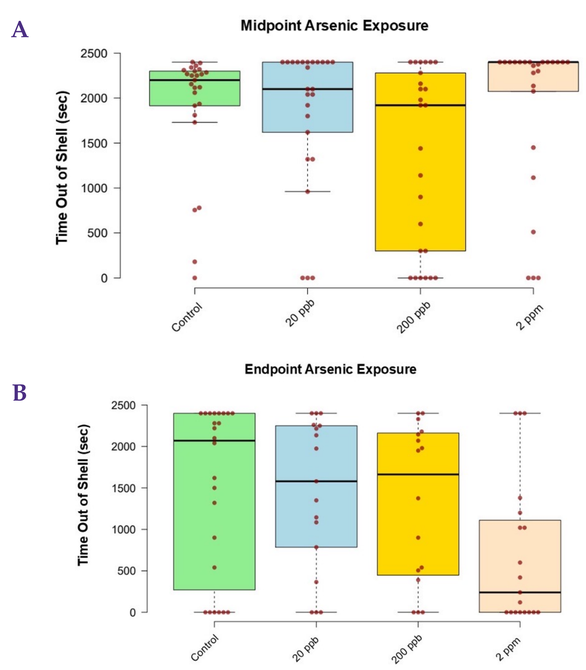


Figure 6: Decrease in activity level from high arsenic exposure not evident until endpoint of treatment. Time spent out of shell was used as a determinant of activity level of the juvenile CMS over a 40-minute period. These graphs show the average time in seconds spent out of shell, with each dot representing individual snails. (**A**) Results of average activity level for each treatment group after 2 weeks of As exposure. For each treatment group, N=25 snails. (**B**) Results of average activity level for each treatment group after 4 weeks of As exposure. Control N=25 snails, 20 ppb [As] N=18 snails, 200 ppb [As] N=17 snails, 2 ppm [As] N=20 snails.

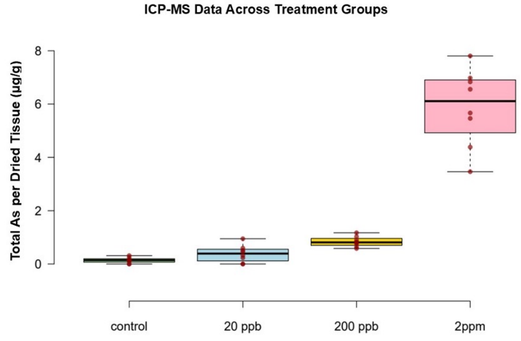


Figure 7: Significant bioaccumulation of arsenic in whole-body tissues in 2ppm As treatment group after 4 weeks. Whole-body snail samples underwent ICP-MS analysis for total [As]. This graph compares average total [As] across treatment groups, with dots representing individual snails. The 2 ppm As group shows a significant increase in bioaccumulation of As relative to the other three groups, with a mean total [As] of 7 $\mu g/g$ dried tissue. For each treatment group, N=8 snails.

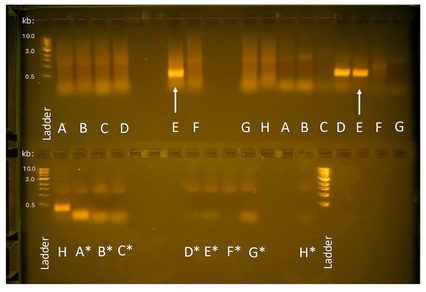


Figure 8: Gel electrophoresis results of 16S rRNA gene amplification. Novel primer pairs were tested for bacterial 16s rRNA amplification to determine suitability for qPCR. The primer pair 331-F and 797-R (labeled 'E') amplified with both low (75 ng/μL) and high (300 ng/μL) [DNA]. A-H represent primer mixes. Each was tested at a high and low [DNA]. Asterisks (*) represent negative controls.

Discussion

- At the endpoint of the 4-week As exposures, there was a relative decrease in mobility (time spend out of shell) across treatment groups, though the 2 ppm As group had the sharpest decline (Fig. 6B). Interestingly, over the course of the 2-week exposure, the 2 ppm As group did not display a decline in mobility (Fig. 6A). The snails in this group remained relatively active.
- ICP-MS analysis (Fig. 7) reveals a significant bioaccumulation of total As in the 2 ppm As group. Though our data does not reveal speciation of As, a high survival and mobility rate as compared to previous research done with adult and sub-adult CMS indicate a plasticity of the juveniles and ability to withstand As toxicity over a short period of time.

Continued Research

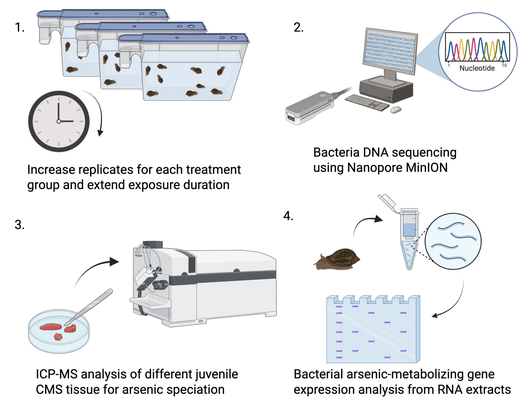


Figure 9: Possible future directions for juvenile CMS research. Increase in treatment replicates would lend greater viability and accuracy of downstream analyses. Increased exposure duration would reveal limitations of juvenile CMS' As toxicity resistance. Nanopore sequencing would uncover microbiome community structure shifts with varying [As] which could be compared to data from adult CMS. ICP-MS analysis of different tissue (i.e. gut, gills, gonads, etc.) for As speciation could tell us how As is being biotransformed and in what organs. RT-PCR for expression of bacterial As-metabolizing genes would further explain As biotransformation processes in juvenile CMS.

Methods

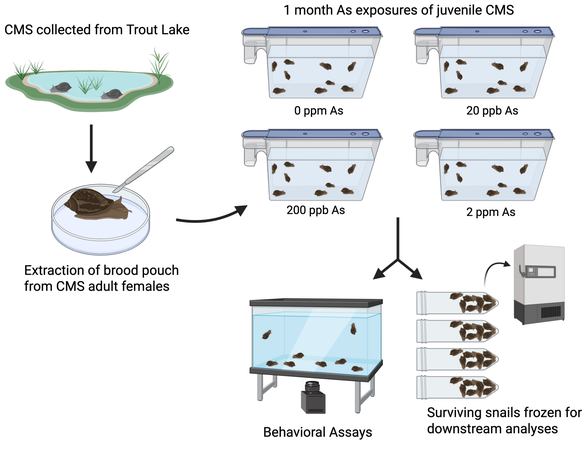
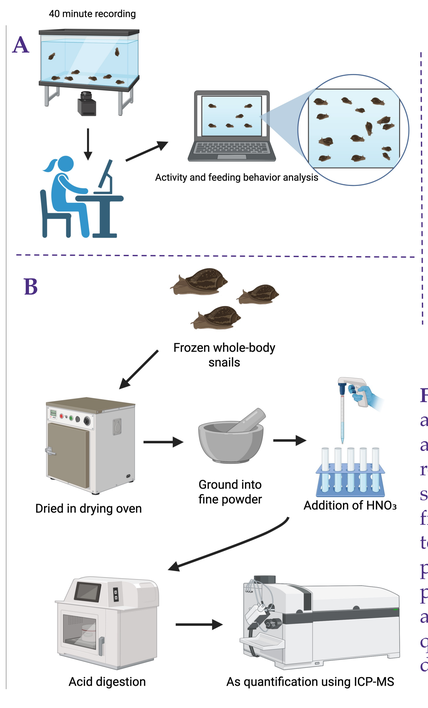


Figure 2: Overview of experimental setup and plan for behavioral and molecular assays. Adult CMS were collected from the Trout Lake, a reference lake with low [As]. Following lab acclimation, brood pouches were extracted from females, and juvenile snails were collected and acclimated to lab conditions. 0 ppm, 20 ppb, 200 ppb, and 2 ppm [As] exposures were conducted for 4 weeks. Behavioral recordings were taken at 2 and 4 weeks. Surviving snails were then frozen at -80°C for further analyses.



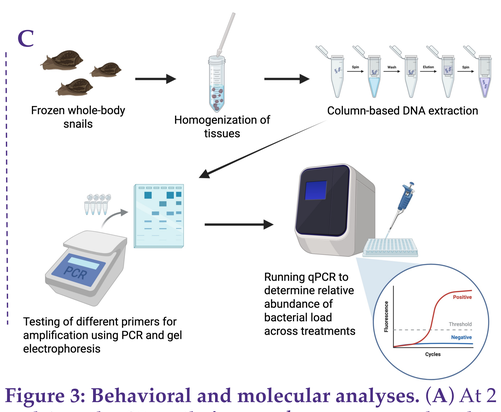


Figure 3: Behavioral and molecular analyses. (**A**) At 2 and 4 weeks, 25 snails from each group were placed in a tank with a camera positioned beneath which recorded for 40 minutes. Researchers tracked time spent out of shell for each snail. (**B**) Whole-body snails from each treatment group were processed for ICP-MS total As quantification. (**C**) Whole-body snails were processed for DNA extraction. 8 different 16S rRNA primer pairs were tested on DNA extracts for amplification. A suitable primer pair was used for qPCR to assess any changes in relative bacterial load depending on treatment group.

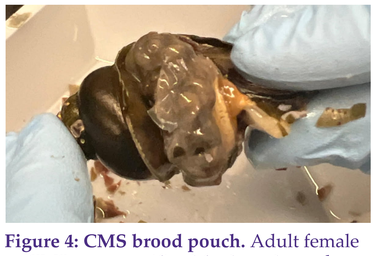


Figure 4: CMS brood pouch. Adult female CMS were sacrificed for brood pouch extraction. Juvenile snails visible beneath tissue layer.

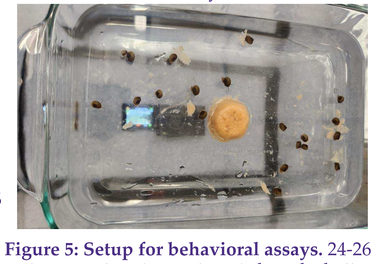


Figure 5: Setup for behavioral assays. 24-26 snails were placed in a glass dish with shallow water and a piece of "snello" for food. A camera was positioned beneath for recording. Note: due to camera troubles cell phone cameras were used in final behavioral assays.

Acknowledgements

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Figure 10: Snail research team. Pictured from left to right: Hannah Seerden, Dr. Sarah Alaei, and Ever Alves.

References