

Investigating the links between arsenic exposure and behavioral impairment in Chinese mystery snails

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Background info:

Arsenic (As) is a legacy contaminant that affects the South Puget Sound region, which accumulates in freshwater ecosystems (e.g. local lakes) and negatively impacts aquatic organisms. With high levels in runoff and shore sediments, this raises the question; how is chronic arsenic exposure in lakes linked to ecosystem health risks? Chinese mystery snails (*Cipangopaludina chinensis*) feed on periphyton, a biofilm of algae and microorganisms that can bioaccumulate arsenic, making dietary exposure a likely route of contamination. The gut microbiome plays an important role in digestion, metabolism, immune function, and communication with the nervous system through the gut-brain axis. This makes them the perfect model organism to observe any biological impacts associated with arsenic exposure in lakes. Previous studies with mice have shown that arsenic exposure can alter microbial community composition. Disruptions to the gut microbiome have been associated with behavioral changes in a variety of animal models (nematodes and mice). Therefore, Chinese mystery snails provide a useful model for investigating whether arsenic exposure influences feeding behavior and whether these behavioral changes may be associated with alterations in the gut microbiome.

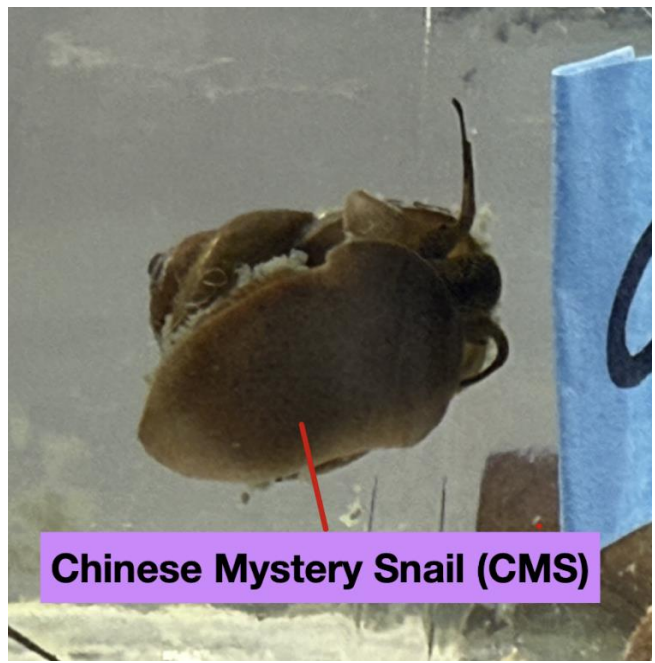
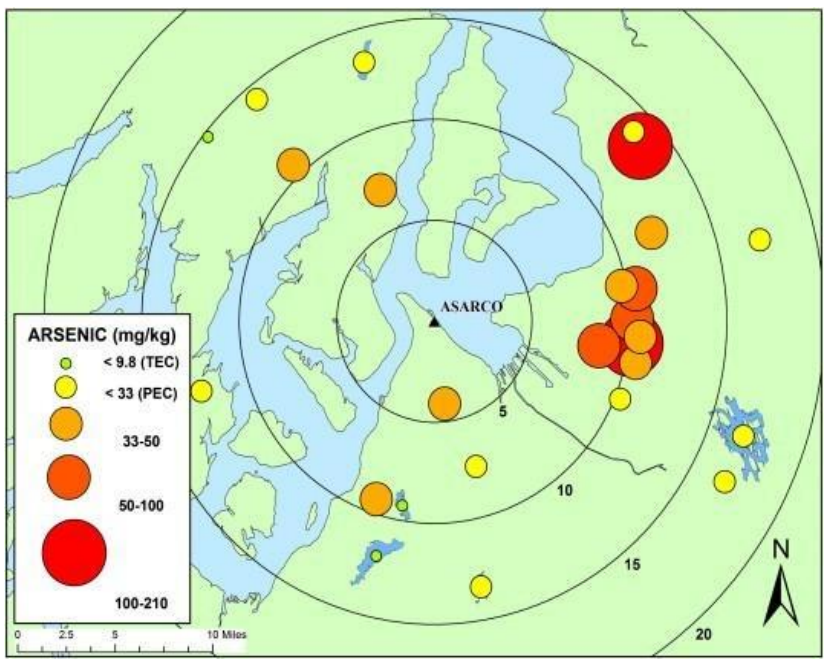


Figure 1: Arsenic Concentrations in South Puget Sound Lake Sediments. Distribution of arsenic concentrations ($\mu\text{g/g}$) in surface sediments from lakes in the South Puget Sound region. Larger circles represent higher arsenic concentrations, with the highest levels detected in lakes near the former ASARCO copper smelter site. Data adapted from Gawel et al., 2014.

Preliminary Findings

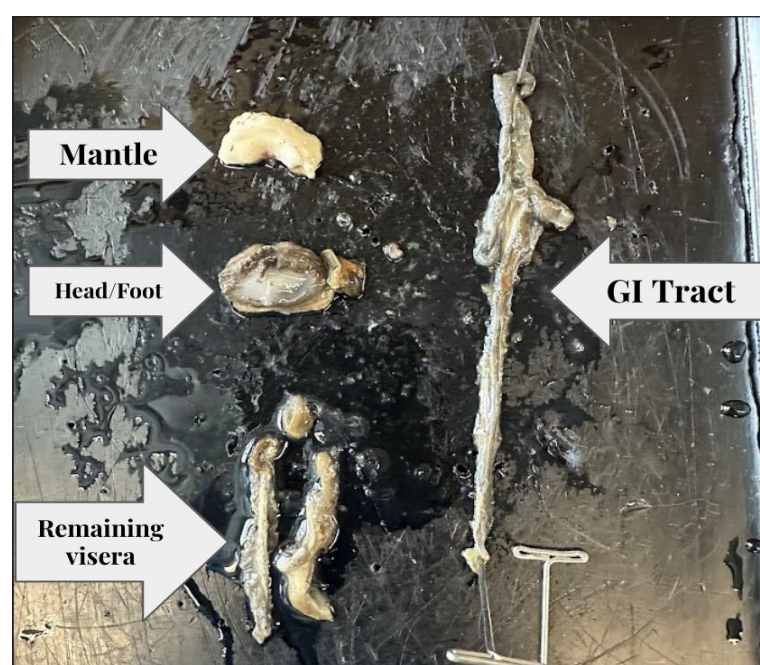


Figure 2a. Bioaccumulation of As located in GI tract and viscera. Dissection and tissue analysis from previous Autumn 2024 lab revealed hyperaccumulation of arsenic in the GI tract and viscera of CMS.

(adapted from Kovalchuk, Rockefeller, Wairimu, et al., manuscript in prep)

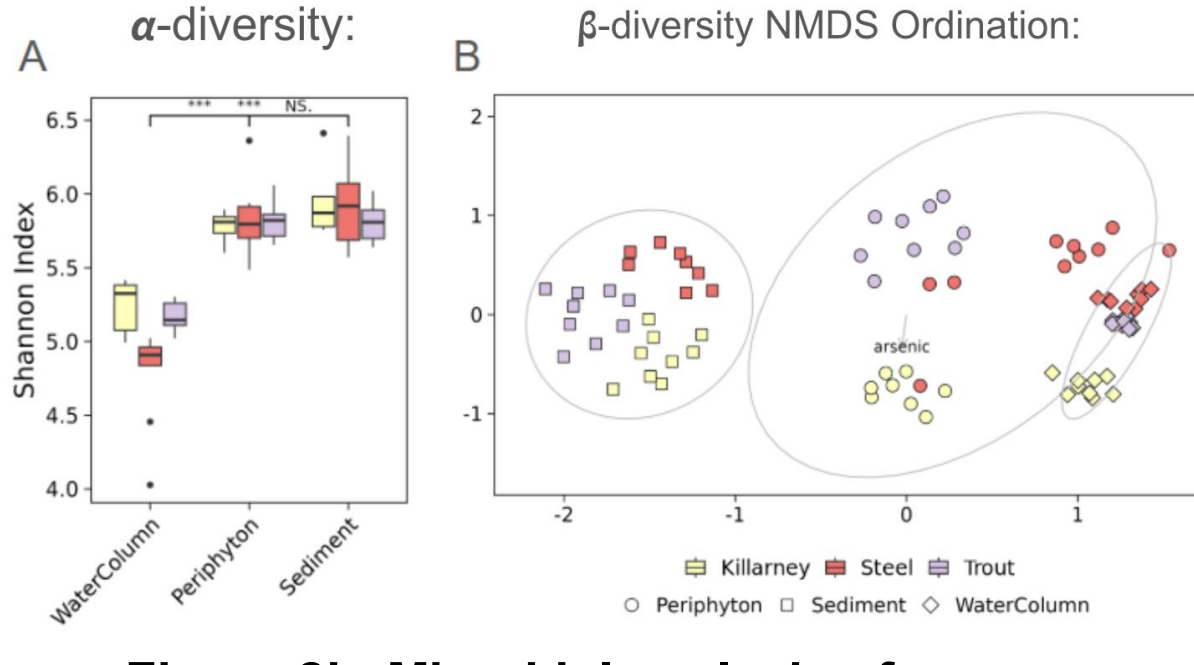


Figure 2b. Microbial analysis of Prokaryotic Communities Revealed Impact of As on Periphyton Composition. 16S rRNA gene barcoding was used to identify and quantify relative abundances of prokaryotic taxa in lake samples. Alpha-diversity (within sample biodiversity) was consistent across lakes. Across-sample comparisons NMDS ordination plot revealed an As-associated shift in periphyton composition

(adapted from Buser, Gardell, Alaei et al.)

Objective

To determine whether acute arsenic [As(V)] exposure alters the feeding behavior, movement, and food preference of Chinese mystery snails by comparing behavioral responses across 0, 0.2, and 0.2ppm arsenic treatments.

Hypothesis

We hypothesized that acute arsenic exposure would alter the feeding behavior of Chinese mystery snails. Specifically, snails exposed to higher concentrations of arsenic would exhibit reduced movement, lower feeding activity, and altered food preference compared with unexposed control snails.

Experimental Design

Experimental Set up for Behavioral and Feeding Assays

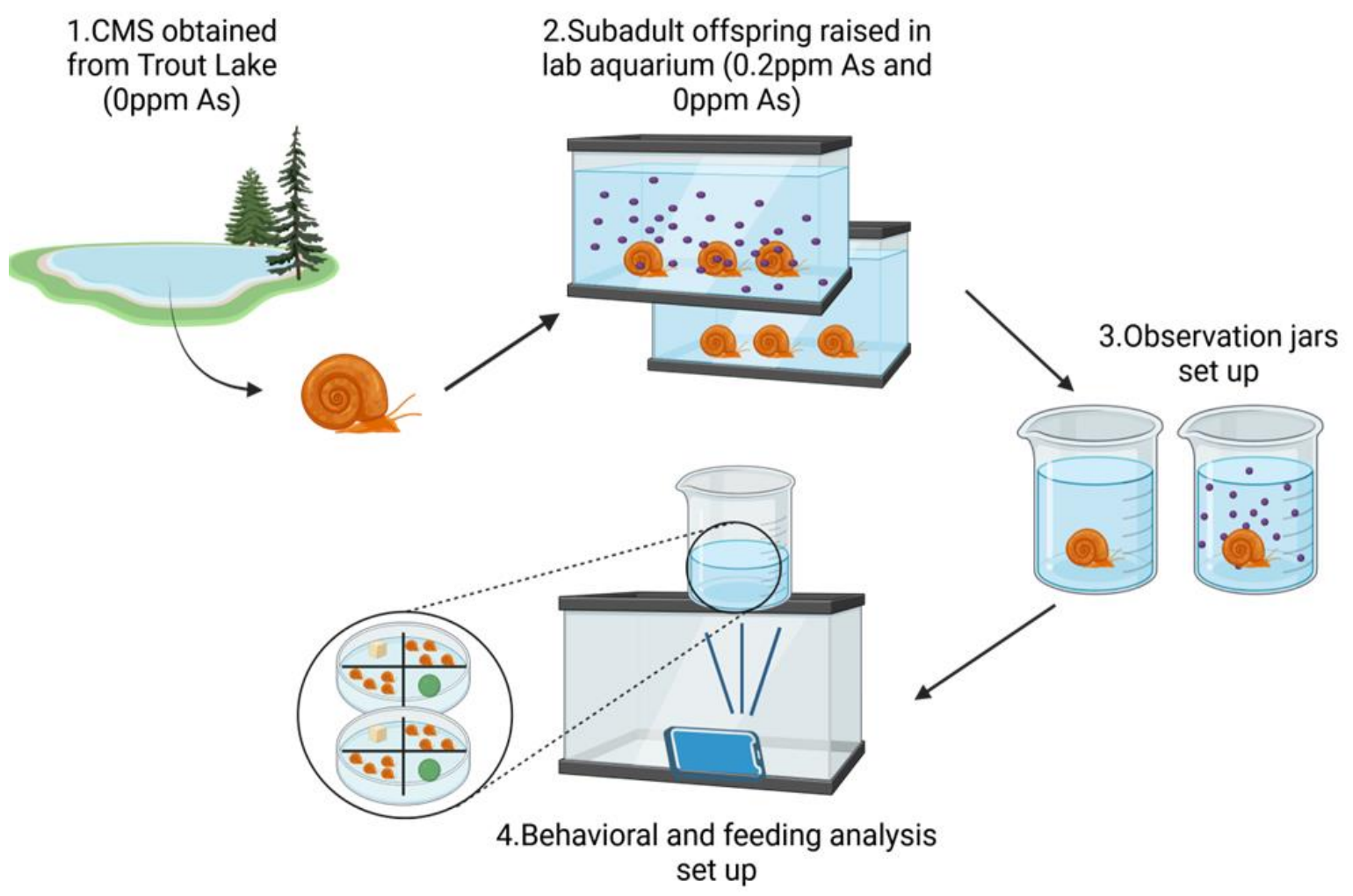


Figure 3: Experimental Setup for Behavioral and Feeding Assays of Chinese Mystery Snails. (1) Chinese mystery snails (CMS) were collected from Trout Lake, a site with low arsenic concentration. (2) Subadult offspring were raised in laboratory aquaria under two treatments: control (0 ppm As) and arsenic-exposed (0.2 ppm As). (3) Observation tanks were prepared for each treatment group. (4) Behavioral and feeding analyses were conducted in quadrants containing alternating snail and food positions to assess potential differences in feeding activity between control and arsenic-exposed snails.

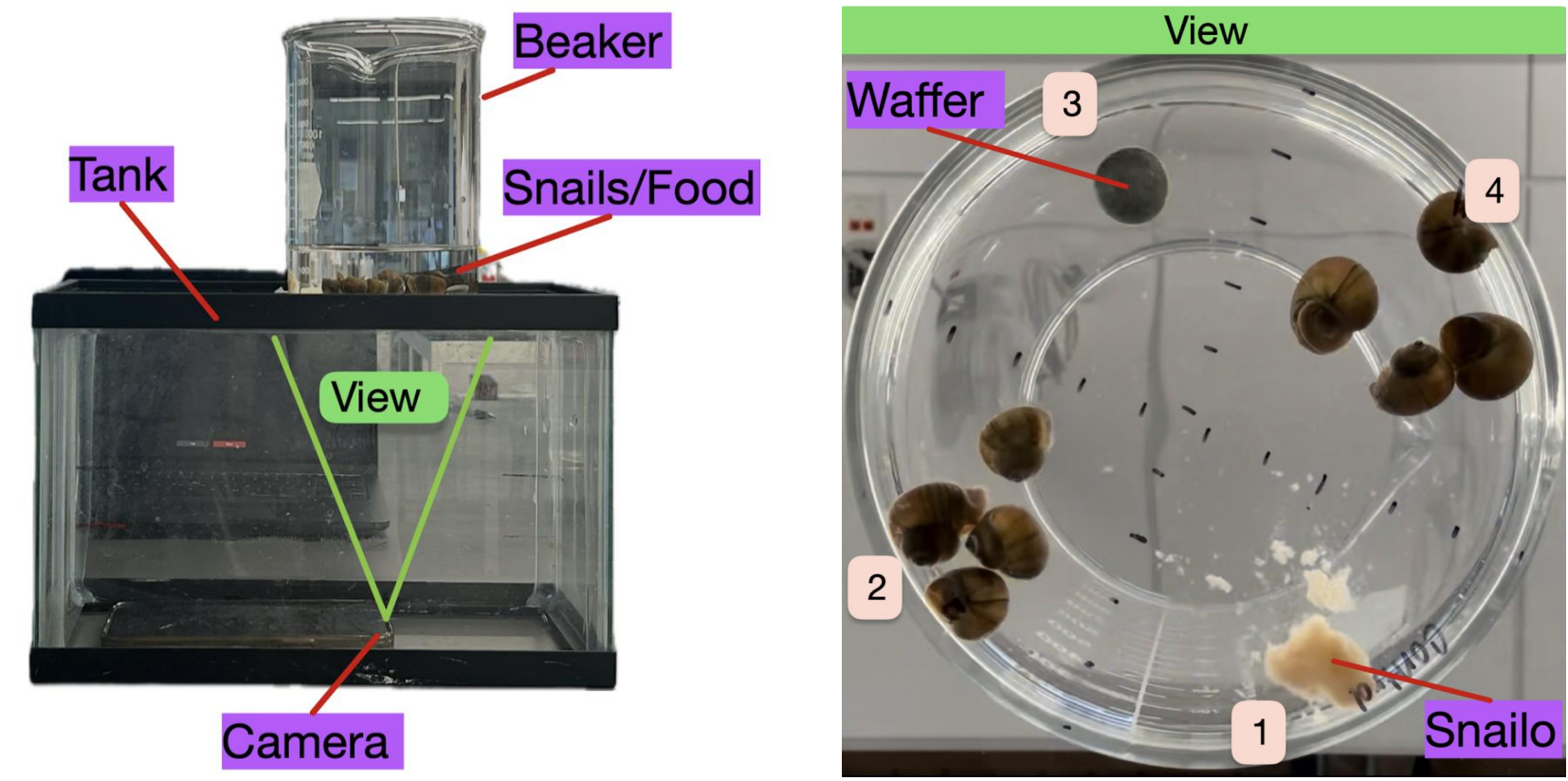


Figure 4: Labeled Behavioral and Feeding Assays Setup and view of quadrants #1-4 (1-snail, 2-snails, 3-waffer, 4-snails).

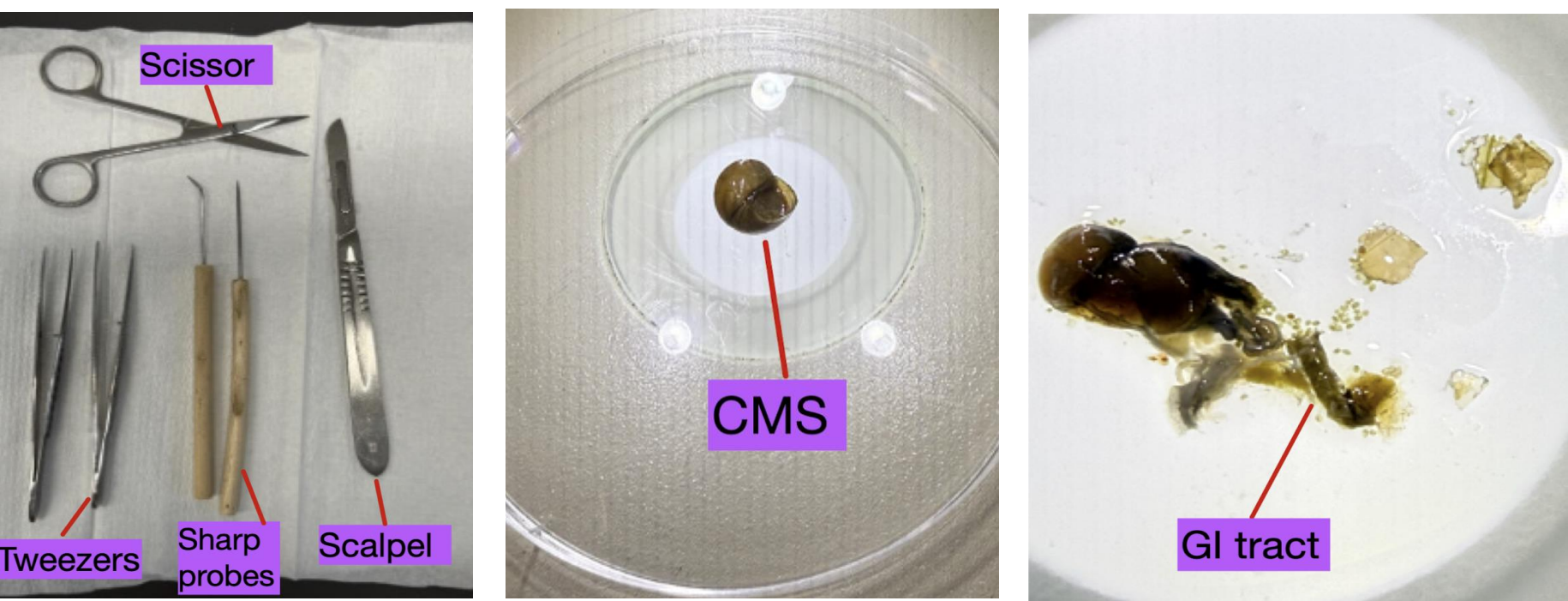


Figure 5: CMS GI tract dissection Setup and image of GI tract. From left to right: dissection instruments used to isolate the gastrointestinal (GI) tract, an intact snail prepared for dissection, and the excised GI tract used for downstream analyses.

Experimental Set up of CMS Gut Microbiome Analysis

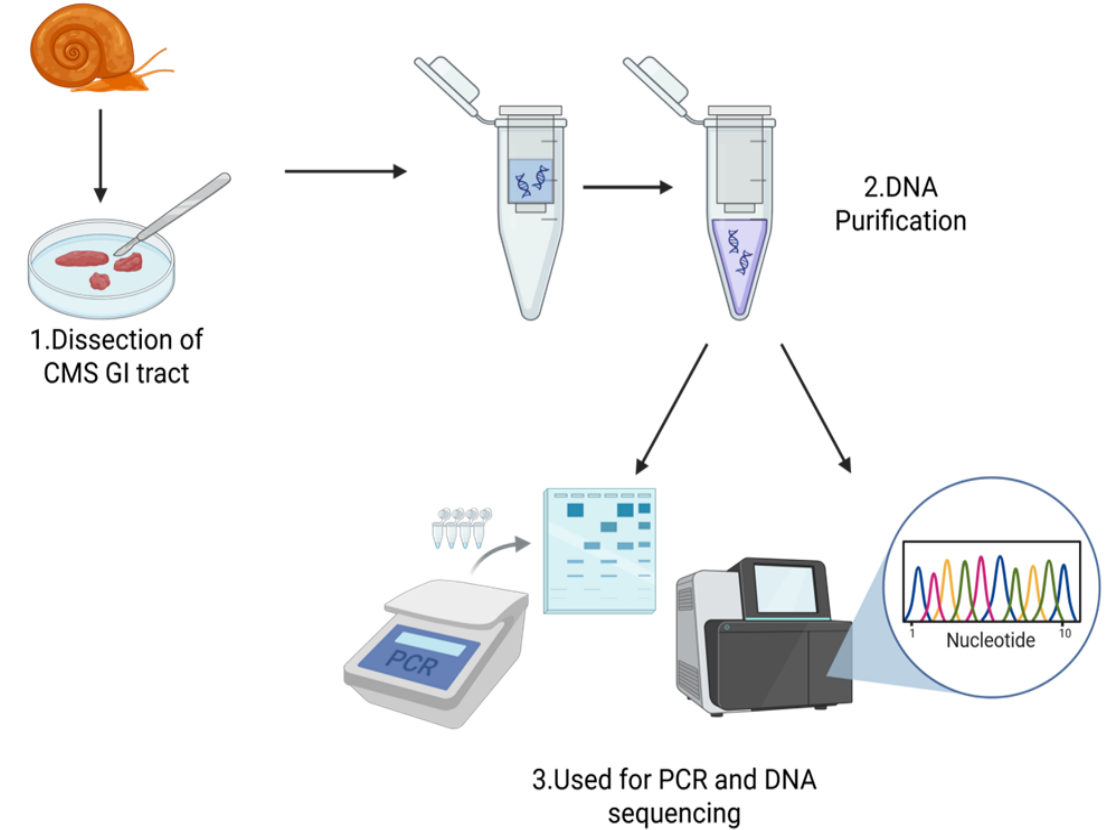


Figure 6: Attempted Experimental Setup for CMS Gut Microbiome Analysis. Workflow showing the process for gut microbiome analysis in Chinese mystery snails. (1) CMS gastrointestinal tracts were dissected, (2) DNA was purified, and (3) samples were used for PCR amplification and DNA sequencing to characterize microbial communities. Due to low concentrations of amplified DNA and time constraints, analysis for the presence of arsenic metabolic genes was implemented (ars B, aio A, arr A, and ars M).

Results

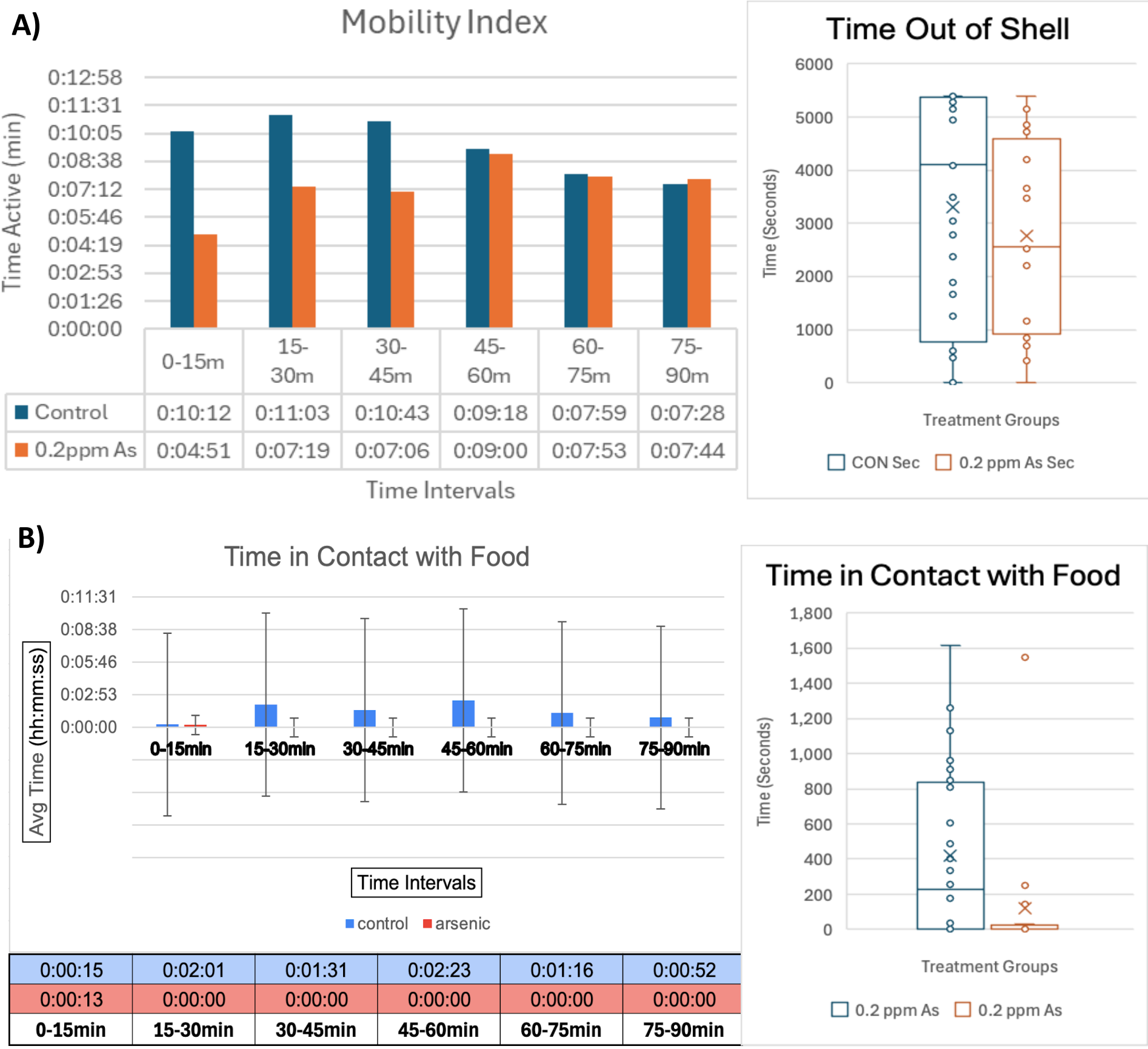


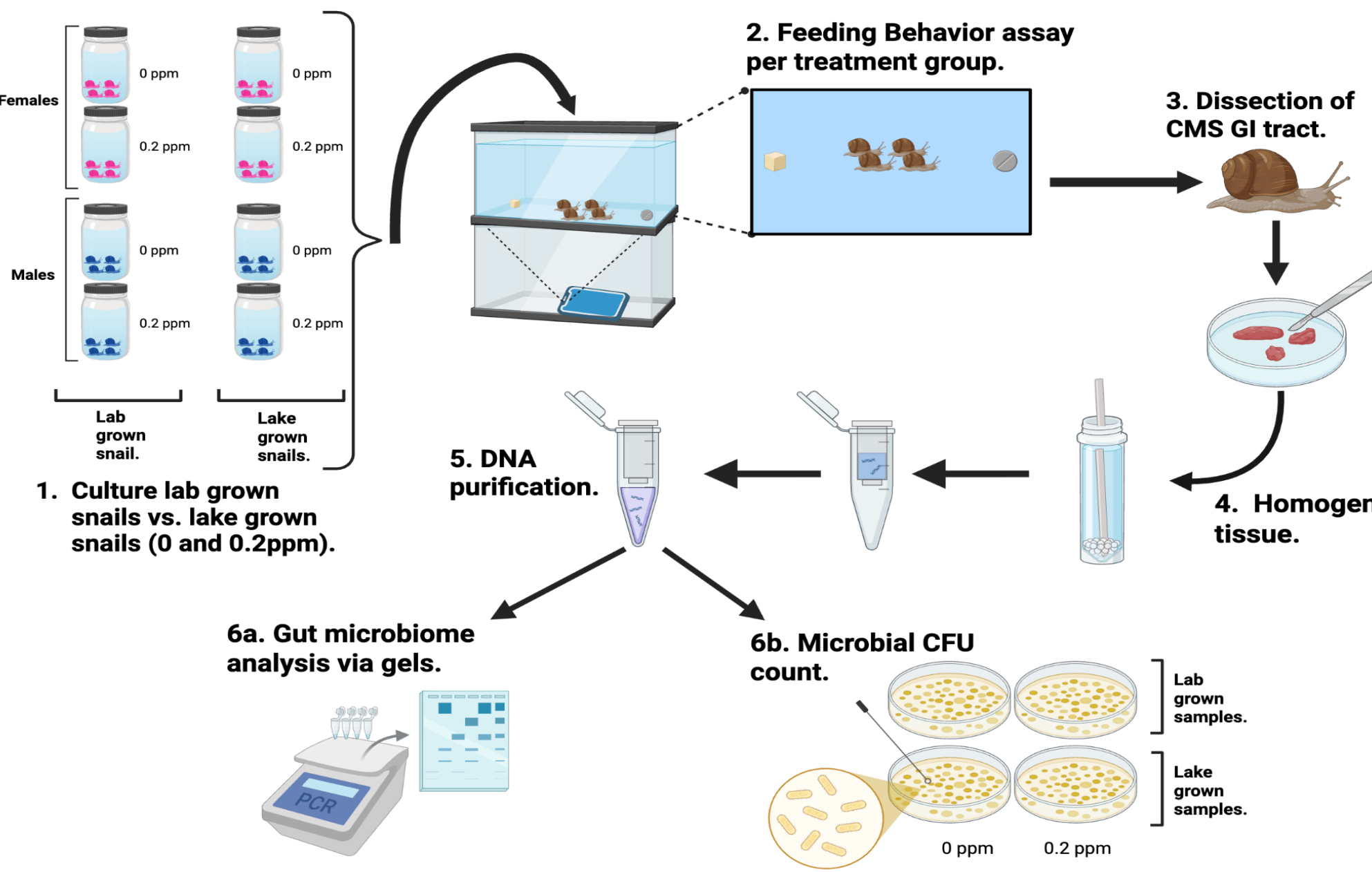
Figure 7a: Delayed Mobility of arsenic-exposed snails. Mobility index (left): control (0 ppm As) and arsenic-exposed (0.2 ppm As) Chinese mystery snails (CMS) were measured across six-time intervals. Arsenic-exposed snails showed a trend of reduced early activity compared to controls, with activity gradually approaching control levels over time. Boxplot showing total mobility time (right): 0.2 ppm arsenic spent less time out of their shell than control snails. P-value 0.25.

Figure 7b: Decreased Feeding Behavior of arsenic-exposed Snails. The average time in contact with food (left): control (0 ppm As) and arsenic-exposed (0.2 ppm As) Chinese mystery snails (CMS) were measured across six time intervals. Arsenic-exposed snails showed a significant reduction of food contact compared to the control treatment group, with only one arsenic-exposed snail making contact in the first 15 minutes. Boxplot showing total feeding time (right): 0.2 ppm arsenic spent significantly less time in contact with food than control snails. P-value 0.02.

Conclusion

Arsenic exposed snails showed a trend in delayed mobility for feeding behavior compared to non-exposed group, along with a significant reduction of contact with food; demonstrating that environmentally relevant arsenic concentrations can alter important behavioral responses. While our findings support the hypothesis that arsenic affects snail behavior, the role of gut microbiome changes in mediating these effects remains unclear due to limited sequencing data. Future studies integrating behavioral assays with comprehensive microbiome analyses will help determine the microbial and neurological mechanisms underlying these behavioral changes.

Future Steps



Future studies will characterize the gut microbiome of laboratory- and lake-exposed Chinese mystery snails to identify bacterial taxa associated with arsenic exposure. Comparing snails collected from lakes with different arsenic concentrations to laboratory-exposed snails will help determine whether observed behavioral changes reflect chronic environmental exposure and identify microbial signatures consistently associated with arsenic bioaccumulation.

Acknowledgements/ References

Special thanks to Dr. Alaei and Dr. Nahmani for their mentorship. The lab staff and my lab partners (Tori J and Tzu T) for their support and contributions to this research.

Lastly, thanks to Salma Wairimu for the encouragement, and previous students/mentors for the preliminary data provided.

