

# Arsenic Exposure Induces Hemocyte Proliferation in Chinese Mystery Snails

Kiana Mueller, Glaysia Sparling, Alison Gardell, Sarah Alaei

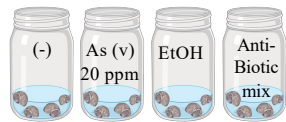


## Introduction

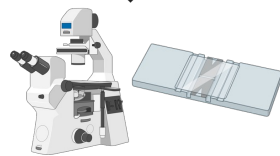
- Local arsenic (As) contamination is found in the Puget Sound area from the ASARCO smelter.
- Invasive Chinese Mystery Snails (CMS) were the organism of study.
- Local residents eat CMS as a part of their diet.
- It's unknown where As accumulates in the CMS, and how it affects the health of the organism.
- When As is detected in the organism as a toxin, the snail produces hemocytes as an immune response
- Tissues of interest include the head/foot, mantle, gut, and everything else.
- HYPOTHESIS 1:** Gut tissue will have higher [As] in contaminated lake than the reference lake.
- HYPOTHESIS 2:** Snails exposed to As treatment will elicit a higher immune response by producing more hemocytes than other treatment groups.
- Understanding which lakes and what parts of a snail are safe to ingest is an important health factor for the residents who eat CMS



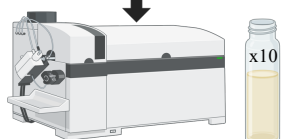
## Methods



- 11-day exposure experiment
- Lab acclimated adolescent snails were used



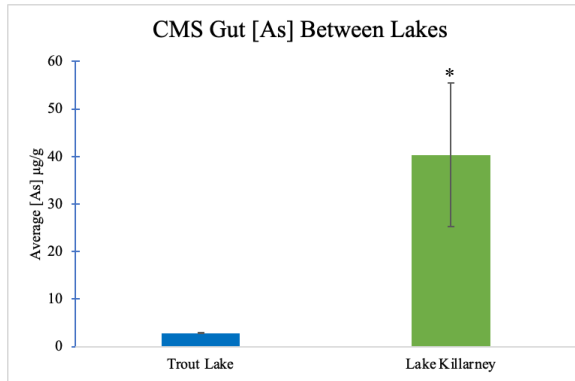
- Hemocytometer was used to count hemocytes



- Inductively coupled plasma mass spectrometry (ICP-MS)
- Field samples were separated anatomically: head/foot, mantle, gut, and everything else

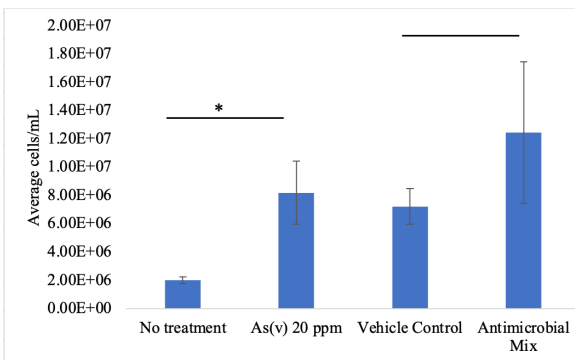
## Results

**Figure 1. Lake Killarney Gut Tissue Sequesters Higher Levels of As Than Trout Lake Gut Tissue**



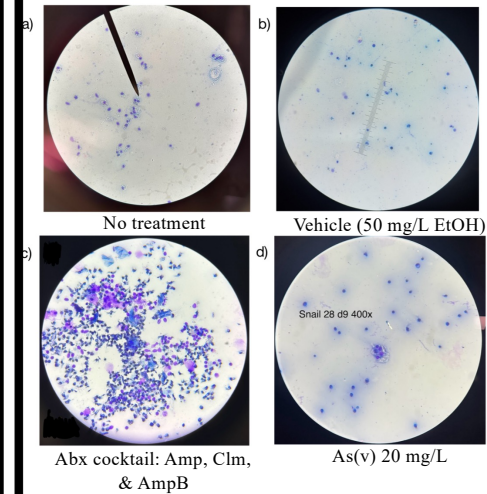
ICP-MS analysis was run on mature male samples (n = 5) collected from the field. a) Average [As] of gut tissue in Trout Lake. b) Average [As] in gut tissue from Lake Killarney. The difference in Lake Killarney from Trout Lake was statistically significant (p = 0.039) when performing a two-tailed, type 2 t-test. Standard error bars represent +/- 1 SEM of [As] in samples.

**Figure 2: As(v) Treatments Caused the Greatest Hemocyte Production**



Day 9 of the exposure treatment sacrificed CMS, and hemolymph was collected. Hemocyte counts were conducted following the Quick Diff protocol. These results suggest that arsenic increases hemolymph cell density significantly (p = 0.023), however, there was no significant trends. The antibiotic treatment seemed to increase the hemolymph cell density more than the other treatments but not significantly supported by the data.

**Figure 3. Hemocyte counts increased after a 9-day exposure treatment.**



Sub-adult CMS were put into four different exposure treatments for 9 days in a mason jar. Each treatment group contained dechlorinated water, 4 CMS, half an algae wafer, and their respective treatments. CMS were sacrificed, hemolymph was collected for hemocyte counts via the Quick Diff kit protocol.

- After confirming As was able to significantly increase the number of hemocytes, we wanted to see where As was accumulating the most, in a natural setting
- Abx treatment may have had higher hemocyte counts due to different counting styles
- Purple cells are indications of granules forming

## Conclusion

- Snail gut tissue bioaccumulates and sequesters higher levels of As(v) than compared to other tissues
- As(v) may be used in place of phosphate for metabolic processes, ∴ we may see more of it in the gut since that's where energy (ATP) is made
- As metabolism genes may integrate into snails' genome via transformation from ingested periphyton
- Abx mix may have caused a larger immune response since those snails died in that treatment group
- Hemocyte counts are indicative of immune response to stress
- Future experiments include exposing periphyton to various levels of As, measure the concentration, and feed that to snails and compare the uptake amounts.

