

# Higher Arsenic Bioaccumulation Observed in Gut Tissue of Male Chinese Mystery Snails in Lake Killarney of South King County, Washington

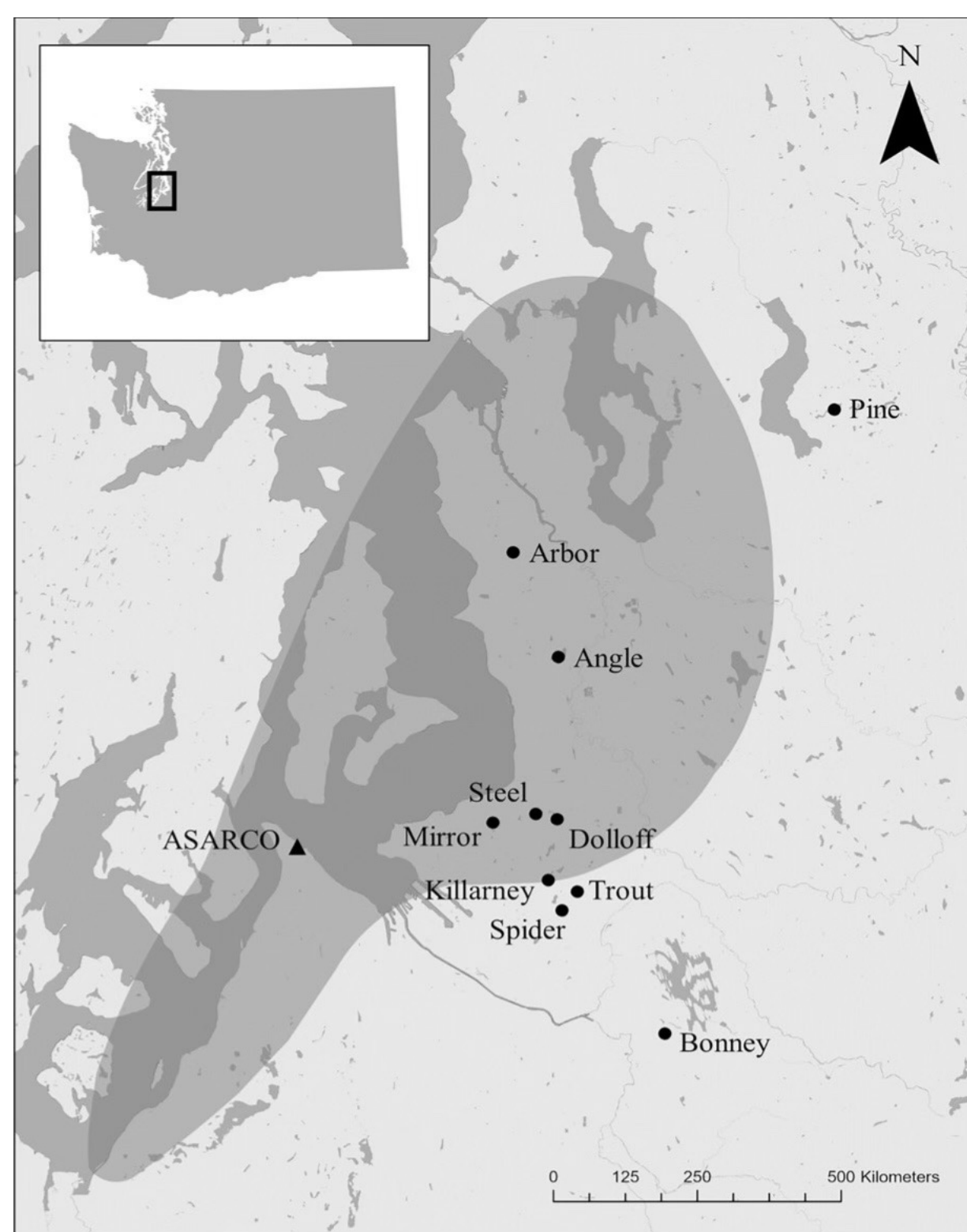
Lyna Man, Nicole Kovalchuk, Dr. Sarah Alaei, and Dr. Alison Gardell  
Division of Sciences & Mathematics | University of Washington Tacoma



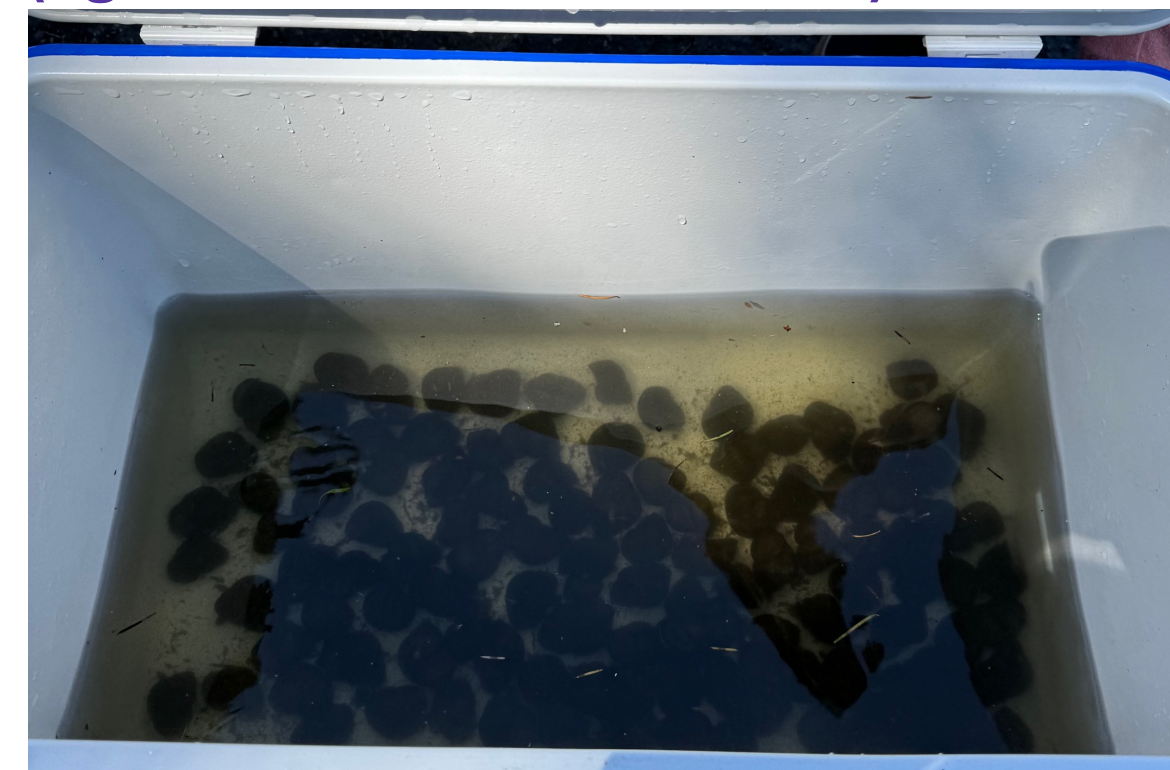
## BACKGROUND

- The ASARCO copper smelting company operated from 1912-1993 in Tacoma, WA and contributed to prolonged exposure of harmful byproducts such as Arsenic (As) to local freshwater lakes.
- Human consumption of aquatic organisms such as Chinese Mystery Snails (CMS) pose an increased risk of cancer.
- CMS diet consists of sediment/underwater surfaces and aquatic organisms such as Periphyton.
- Shallow lake sediment and periphyton are known to accumulate high concentrations of As (Gawel et al. 2023).
- Little is known about the mechanisms behind bioaccumulation of As contamination in CMS.
- The aim of this project was not only to determine the accumulation of As in varying tissue regions but to also investigate CMS exposure to As to further understand the mechanisms behind As contamination in local freshwater ecosystems.

## Affected Regions and Field Samplings



**Fig. 1:** The figure depicts a map of the local lakes in Tacoma, WA. The grey shaded region represents the Lakes which were impacted by the As deposition from the smelter's plume. (figure from Gawel et al. 2023)

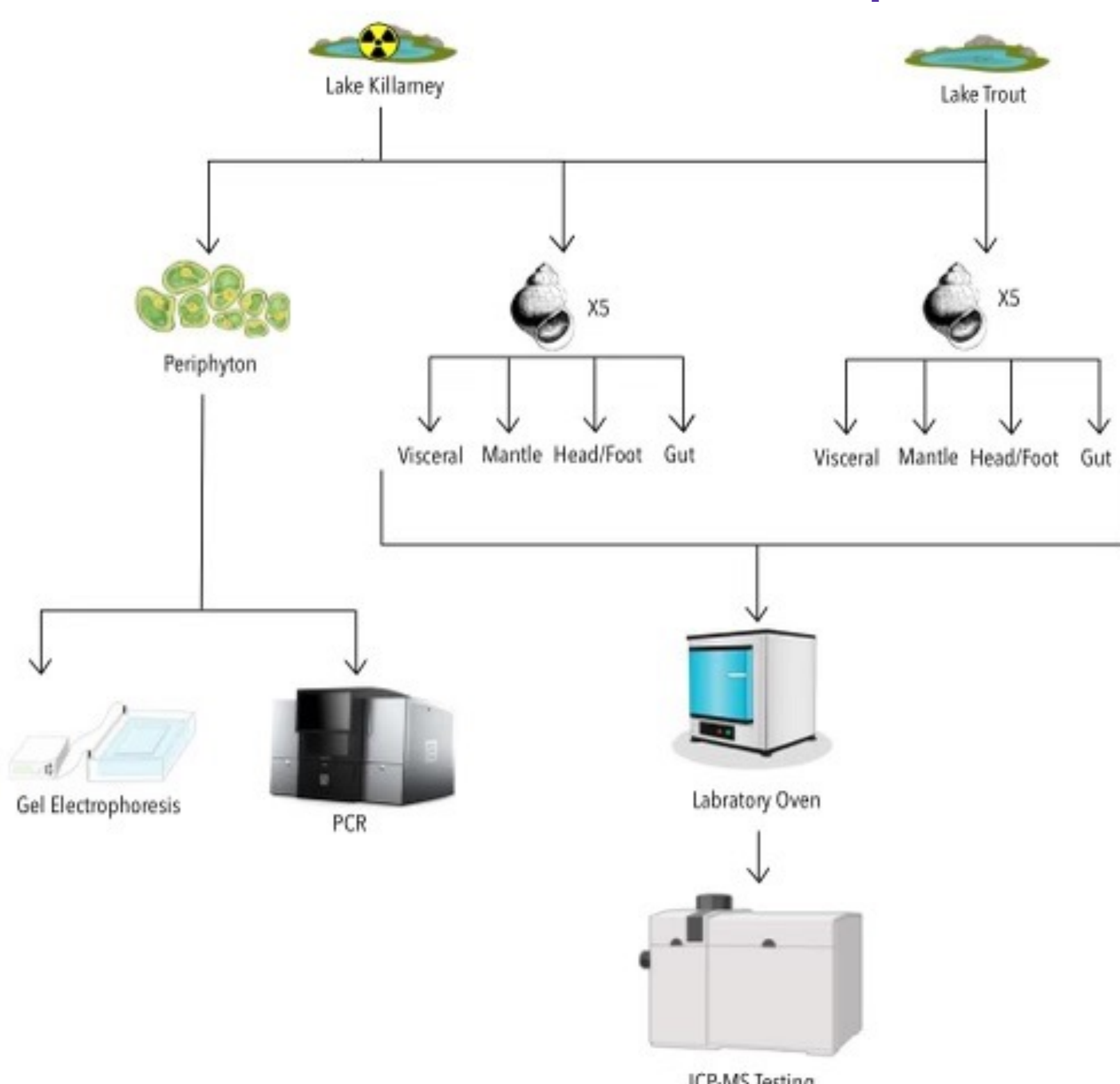


**Fig. 2:** Our research group went on a field day where we experienced harvesting the CMS ourselves. Above is a picture of the snails we collected. (self-sourced)

## OBJECTIVE/METHODS

- Quantified As content in male CMS of 4 tissues regions such as the gut, head/foot, mantle, and visceral regions from two lakes:
  - Lake Killarney (As contaminated)
  - Lake Trout (Reference lake)
- Test for presence of detoxification gene *arsM*, in periphyton of Lake Killarney, Lake Steel, and Lake Trout.
- HYPOTHESIS: Bioaccumulation of As will be higher in gut tissue of CMS from Lake Killarney. Furthermore, higher amounts of *arsM* would be detected in Lake Killarney relative to Lake Steel and Trout.**

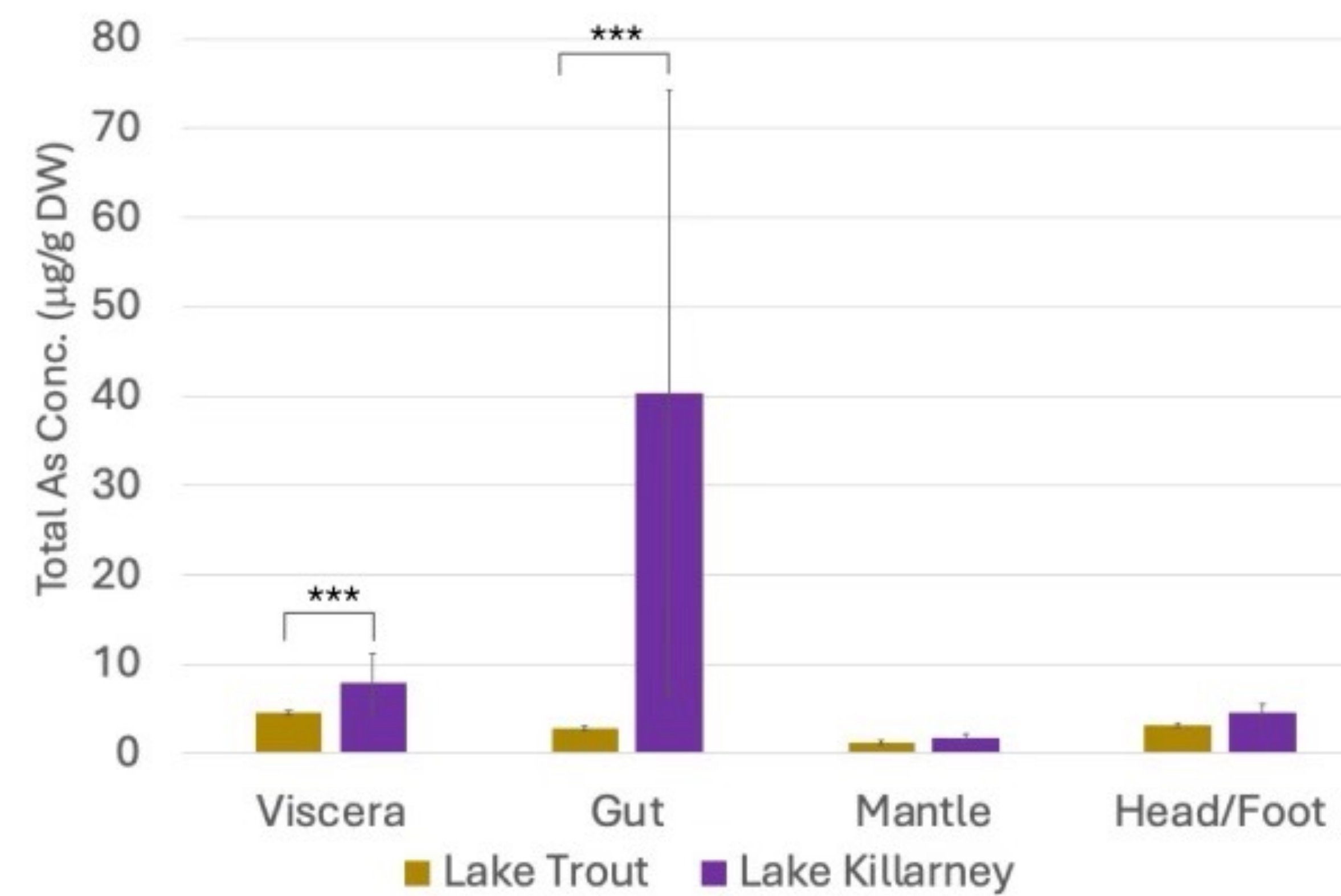
## Process of Experimentations



**Fig. 3:** Schematic which details the process used to observe the accumulation of As in CMS tissues. Five CMS samples from each lake were dissected and separated into 4 different tissue types. Quantification of As content in the tissue regions were determined through ICP-MS. Periphyton collected from Lake Killarney, Lake Trout, and Lake Steel underwent PCR with 16s rRNA (control) and *arsM* primers. Presence of ABG *arsM* was measured through gel electrophoresis.

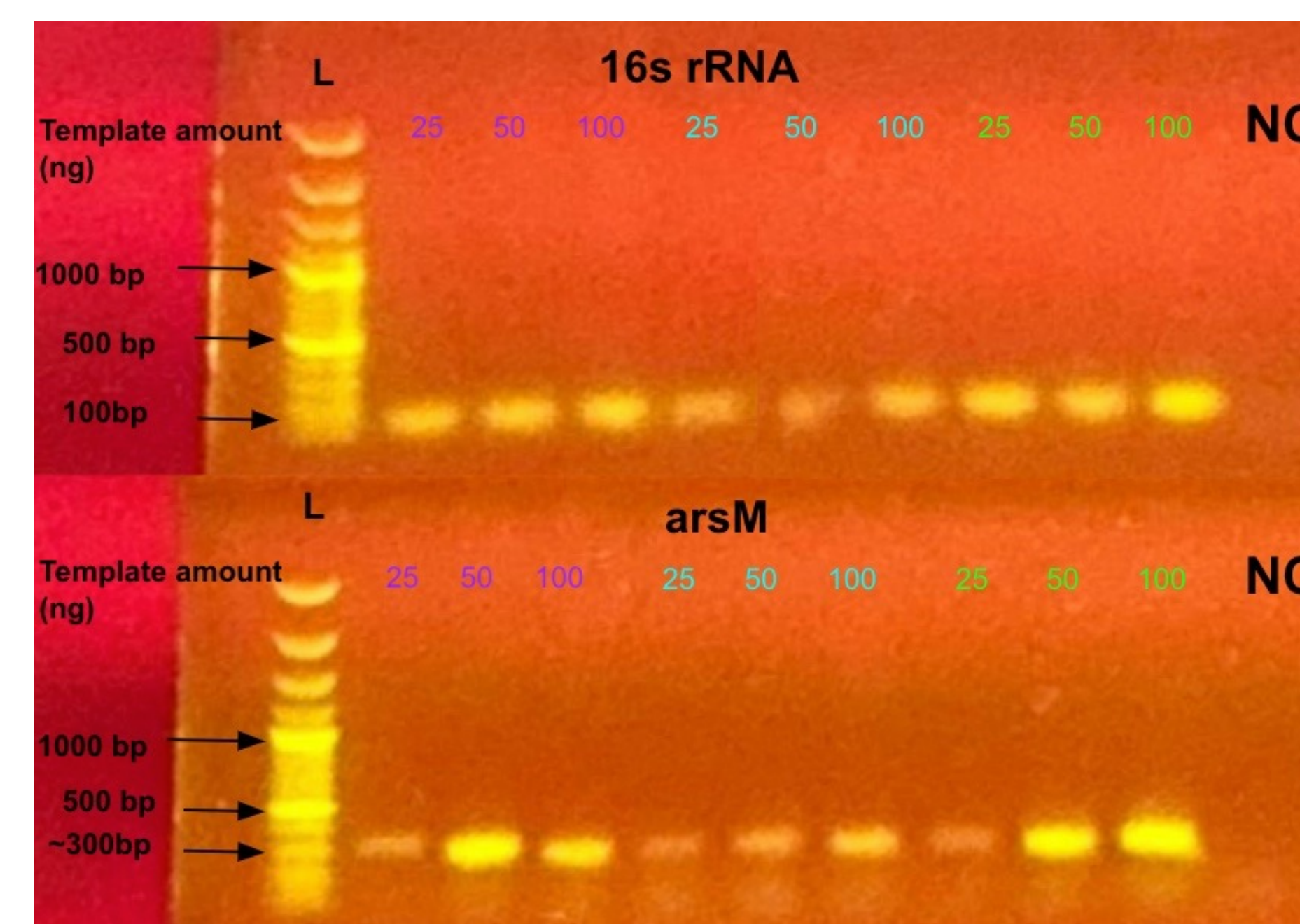
## RESULTS

### Arsenic Bioaccumulation in Male CMS Tissue from Lake Trout



**Fig. 4: Arsenic accumulates most in gut tissue of CMS from Killarney Lake.** As accumulation in various tissues of male CMS from both Lake Killarney and Trout Lake. Trout Lake (<1 ppb As in H<sub>2</sub>O) is the control lake in this experiment. This data was collected through a study where adult male CMS snails (n=5) from each lake were dissected by tissue region. Tissue regions are categorized as the head/foot, gut, mantle, and visceral tissues which after separation were dried. After the drying process, the tissue pieces are then grinded into a fine powder and digested through ICP-MS prep with nitric acid. After testing the samples with ICP-MS, As accumulation data was run through single-factor ANOVA indicating significant difference between As accumulation in sections of CMS tissue. This figure is a visualization of the amount of As in the gut, mantle, head/foot, and visceral tissues. \*\*\*=p<0.001

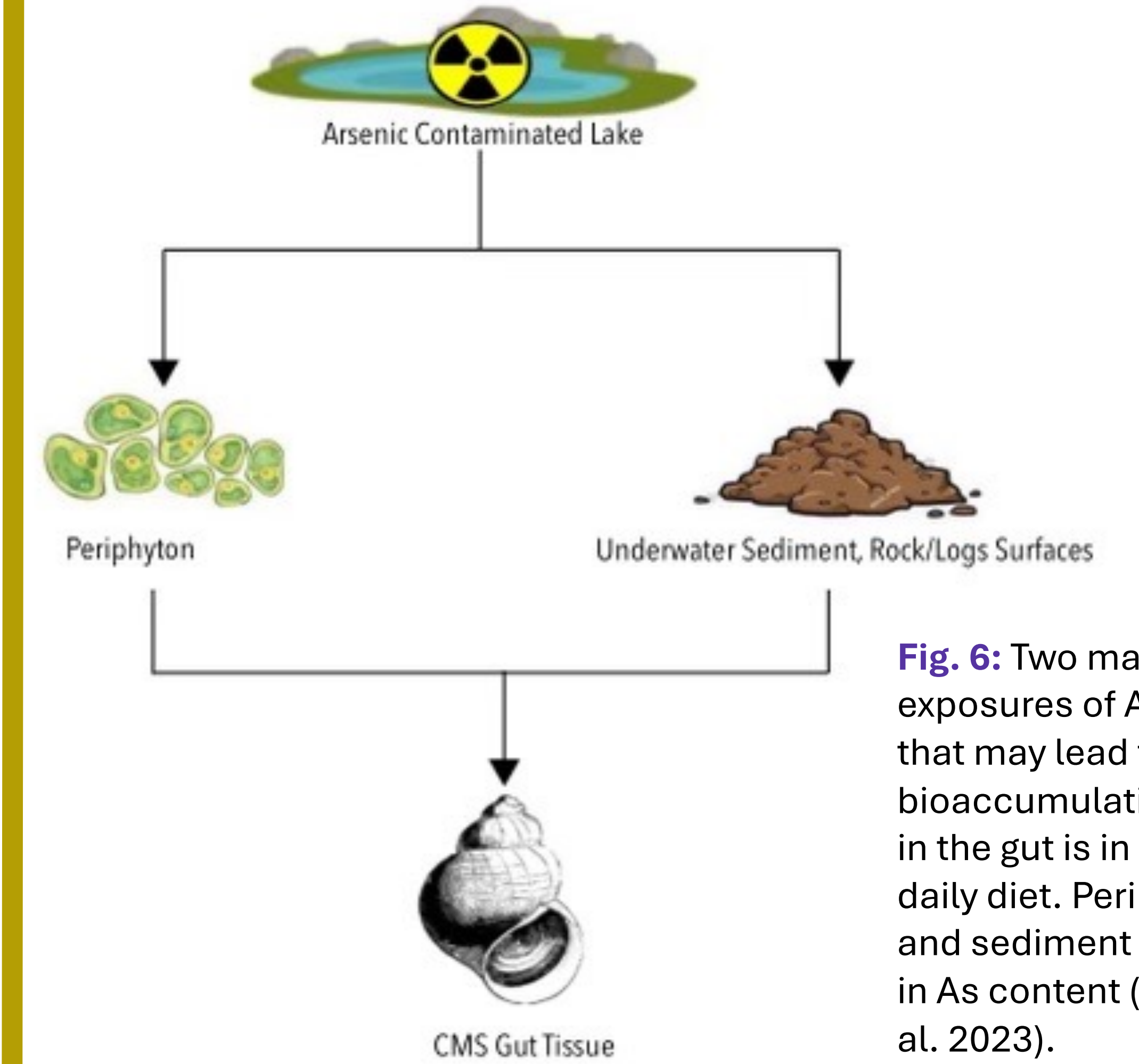
### Higher Presence of *arsM* in Periphyton from Lake Killarney



**Fig. 5: The presence of enzyme that encodes for Arsenite methylase (*arsM*) was detected in periphyton DNA.** Periphyton samples were categorized by lake and amount of DNA template added in PCR reaction. Purple=Trout Lake (<1 ppb As in H<sub>2</sub>O), Teal=Steel Lake (2 ppb As in H<sub>2</sub>O), and Green=Lake Killarney (20 ppb As in H<sub>2</sub>O). Primer set 16s rRNA was used as a positive control. Primer set *arsM* was used to test if *arsM* was present in periphyton DNA. 16s rRNA demonstrated bacterial DNA exists within the periphyton DNA samples. *ArsM* primer results indicate presence of *arsM* gene in periphyton. Lake Killarney with 50 ng, 100 ng, and Trout with 50 ng qualitatively had the most abundant presence of *arsM*. The two negative controls (NC) indicate no cross contamination.

## DISCUSSION/FUTURE STEPS

### Is Dietary Exposure of High As Contributing to As Bioaccumulation in Gut Tissues?



**Fig. 6:** Two main exposures of Arsenic that may lead to higher bioaccumulation of As in the gut is in CMS daily diet. Periphyton and sediment are high in As content (Gawel et al. 2023).

### Results:

- Data showed high As bioaccumulation in gut tissue from Lake Killarney relative to all other tissues in both lakes.
- Results of periphyton *arsM* detection and high As bioaccumulation in gut tissue shows a connection between CMS high As diet and As concentration in gut.

### Future Steps:

- The presence of detoxification mechanisms in periphyton leads us towards exploring the presence of ABGs in the gut bacteria of CMS, to determine if CMS have detoxification mechanisms due to prolonged exposure as well.
- Experimenting with CMS diet, contaminated sediment and periphyton vs. control diet and test for As accumulation in various tissue regions, most importantly gut tissue.
- Continued research regarding ABGs in periphyton as a method to detoxify Arsenite in contaminated lakes.
- Lastly it might be beneficial to replicate this experiment with a greater sample size, especially for the gut. Error bars on our graph showed a high range of variation in gut data which could be better understood with a follow up experiment.

## ACKNOWLEDGEMENTS

- I would like to thank Dr. Sarah Alaei and Dr. Alison Gardell for their mentorship and support throughout this 10-week long project. Their mentorship gave me the confidence in myself to believe that I have the qualities necessary to work in a professional lab environment.
- I would also like to thank all my peers in TBIOL 495. Their ability to work so well under pressure and strict time frames with a smile on their faces greatly helped our research move forward.

## REFERENCES

- Gawel et al. 2023. Littoral sediment arsenic concentrations predict arsenic trophic transfer and human health risk in contaminated lakes. PLOS ONE. 18(10):e0293214. <https://doi.org/10.1371/journal.pone.0293214>
- Wang et al. (2023). Deciphering roles of microbiota in arsenic biotransformation from the earthworm gut and skin. Journal of Hazardous Materials. 446:130707. <https://doi.org/10.1016/j.jhazmat.2022.130707>