# Arsenic Toxicity: Chinese Mystery Snail (CMS) Bioaccumulation and Periphyton Biotransformation Genes

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INTRODUCTION

Arsenic (As) is a metalloid contaminant that disrupts many physiological pathways resulting in neurotoxic, cytotoxic, and mutagenic effects. Due to 100 years of ASARCO smelter activity and demolition in Ruston, WA, many surrounding freshwater lakes within the deposition zone are contaminated with As.
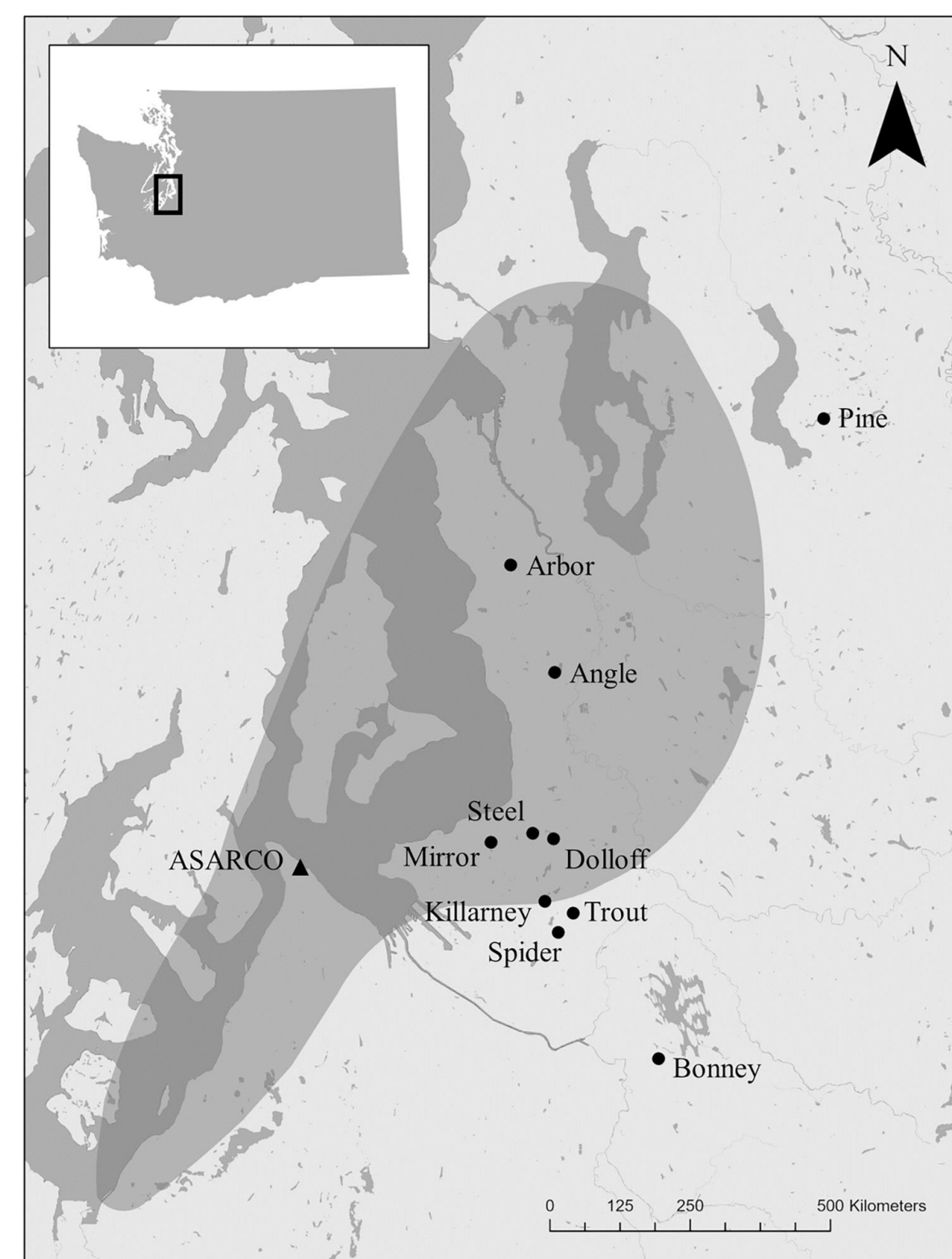
Trout Lake
- Sediment: 7 µg As g-1
- Periphyton: 25±3.2 µg As g-1

Steel Lake
- Sediment: 50±26.5 µg As g-1
- Periphyton: 128±6.7 µg As g-1

Lake Killarney
- Sediment: 213±84 µg As g-1
- Periphyton: 627±92 µg As g-1

Figure 1: **Smelter deposition zone resulting from ASCARO plume** adapted from Hull et al. 2023.
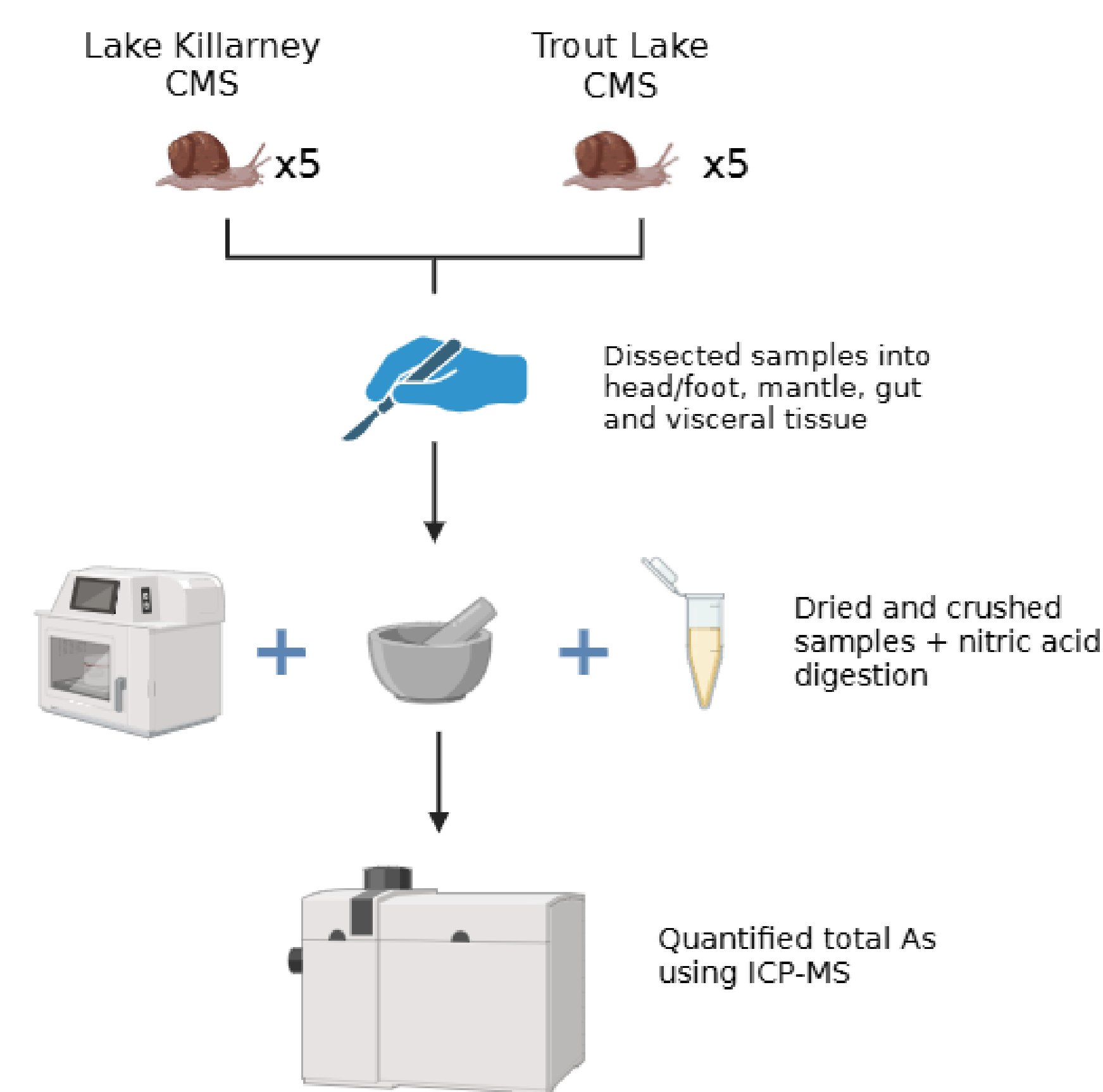
Bioaccumulation is the process in which pollutants are absorbed by a living organism through direct contact or dietary intake faster than it can be removed (Nnaji et al. 2023). Given that locals live, swim, and fish out of these lakes, investigating aspects of potential bioremediation mechanisms is important in addressing As toxicity on ecological health.
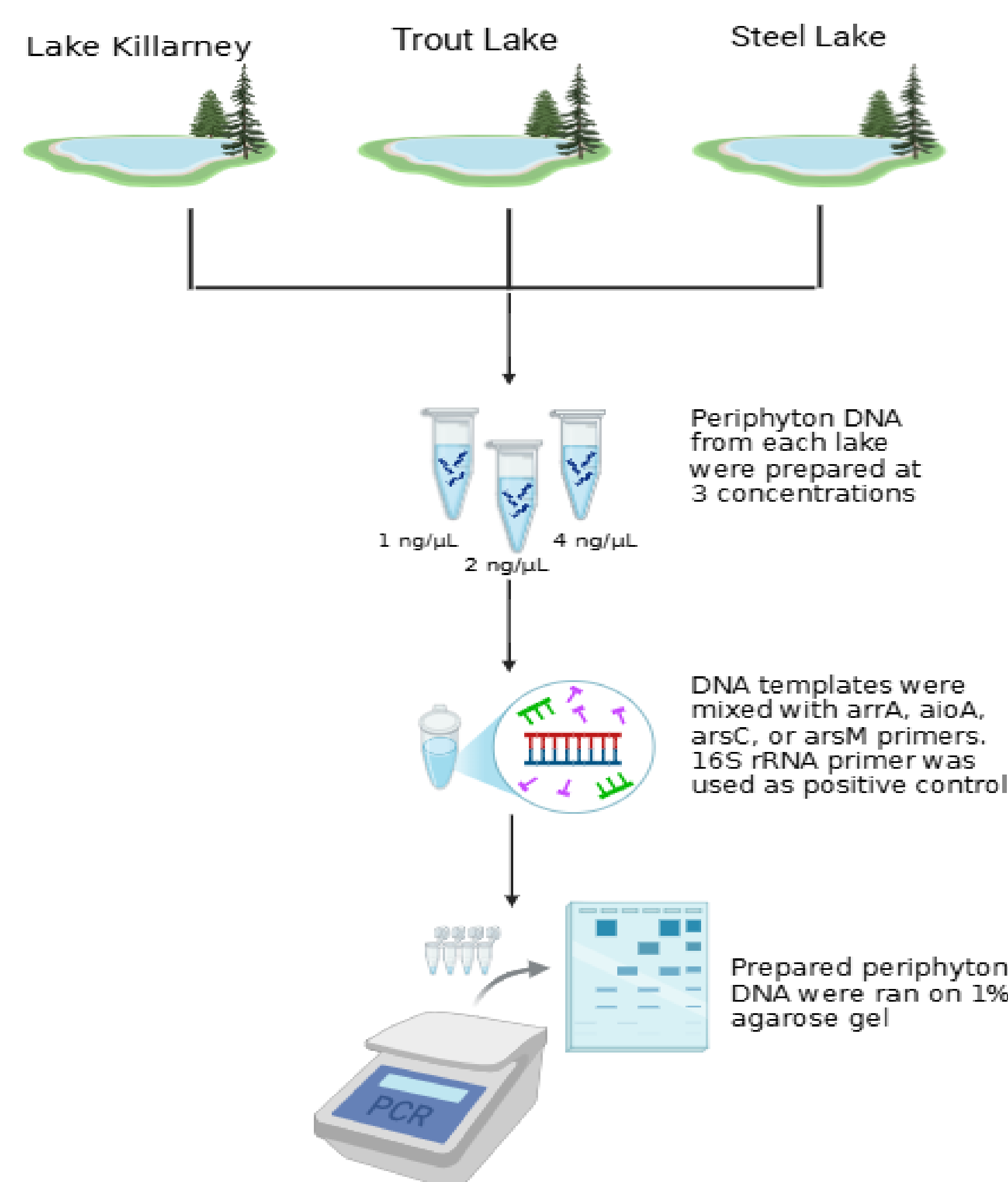
OBJECTIVE/HYPOTHESIS

o **Objective:** Determine whether or not Arsenic bioaccumulates differently across CMS head/foot, mantle, gut, and viscera and identify the presence of periphyton As biotransformation genes.

o **Hypothesis:** Arsenic concentrations will be highest in the gut due to direct consumption of As-rich periphyton and As biotransformation genes will be present in periphyton DNA.

METHODS

Arsenic Bioaccumulation Analysis

Lake Killarney CMS x5   Trout Lake CMS x5

Dissected samples into head/foot, mantle, gut and visceral tissue

Dried and crushed samples + nitric acid digestion

Quantified total As using ICP-MS

Periphyton Biotransformation Analysis

Lake Killarney   Trout Lake   Steel Lake

Periphyton DNA from each lake were prepared at 3 concentrations
1 ng/µL   2 ng/µL   4 ng/µL

DNA templates were mixed with arrA, aioA, arsC, or arsM primers. 16S rRNA primer was used as positive control.

Prepared periphyton DNA were ran on 1% agarose gel

RESULTS

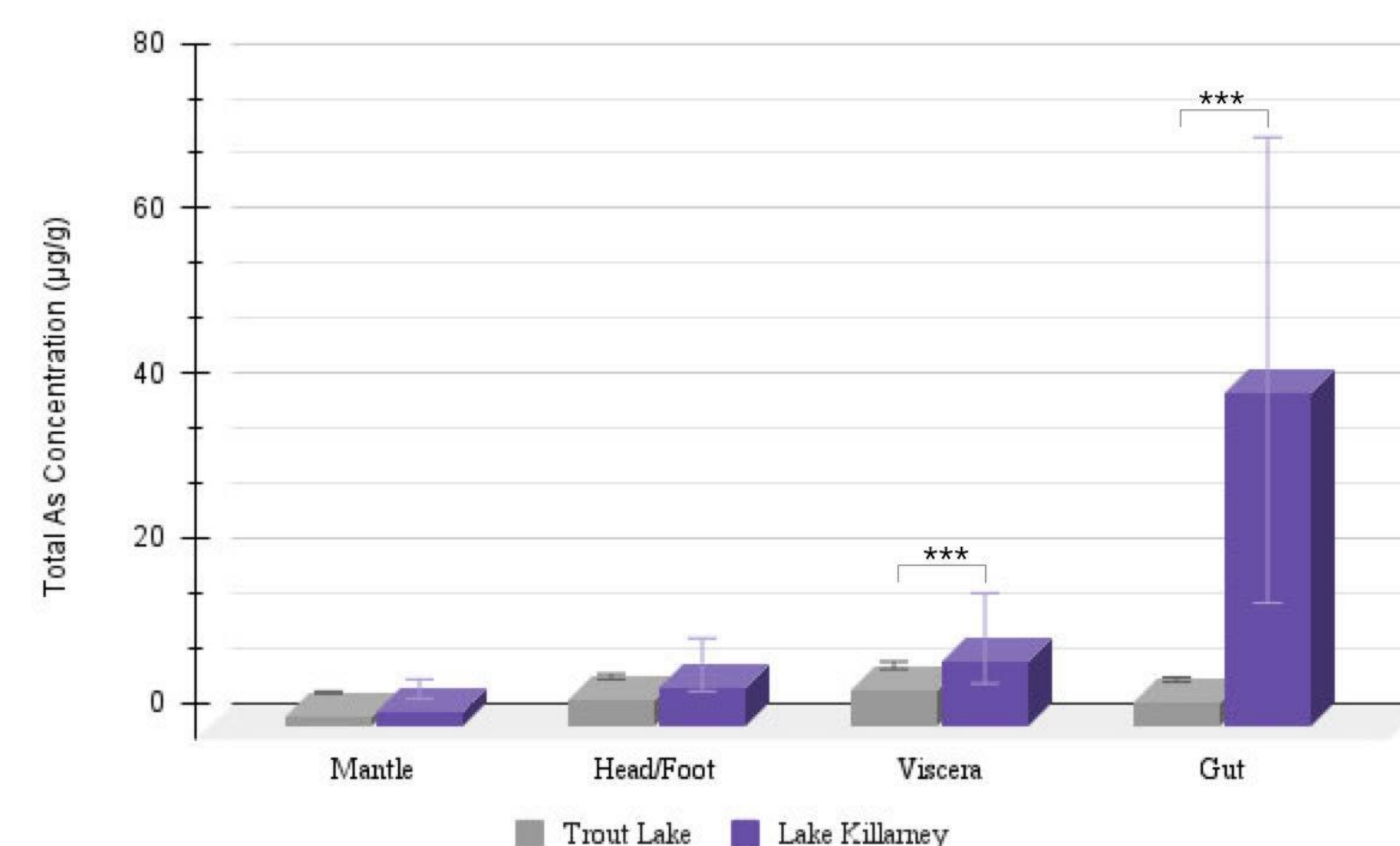### Arsenic Bioaccumulates Highest in the Gut of CMS

Figure 2: **Significantly higher concentrations of As observed in the gut of male CMS from Lake Killarney.** Four tissue regions (mantle, head/foot, gut, and viscera) were dried, digested, and analyzed using ICP-MS to quantify total arsenic concentration in n=5 for each lake. This graph represents a single factor ANOVA depicting how As bioaccumulates across tissues in snails from Trout Lake and Lake Killarney. ***=$p < 0.001$

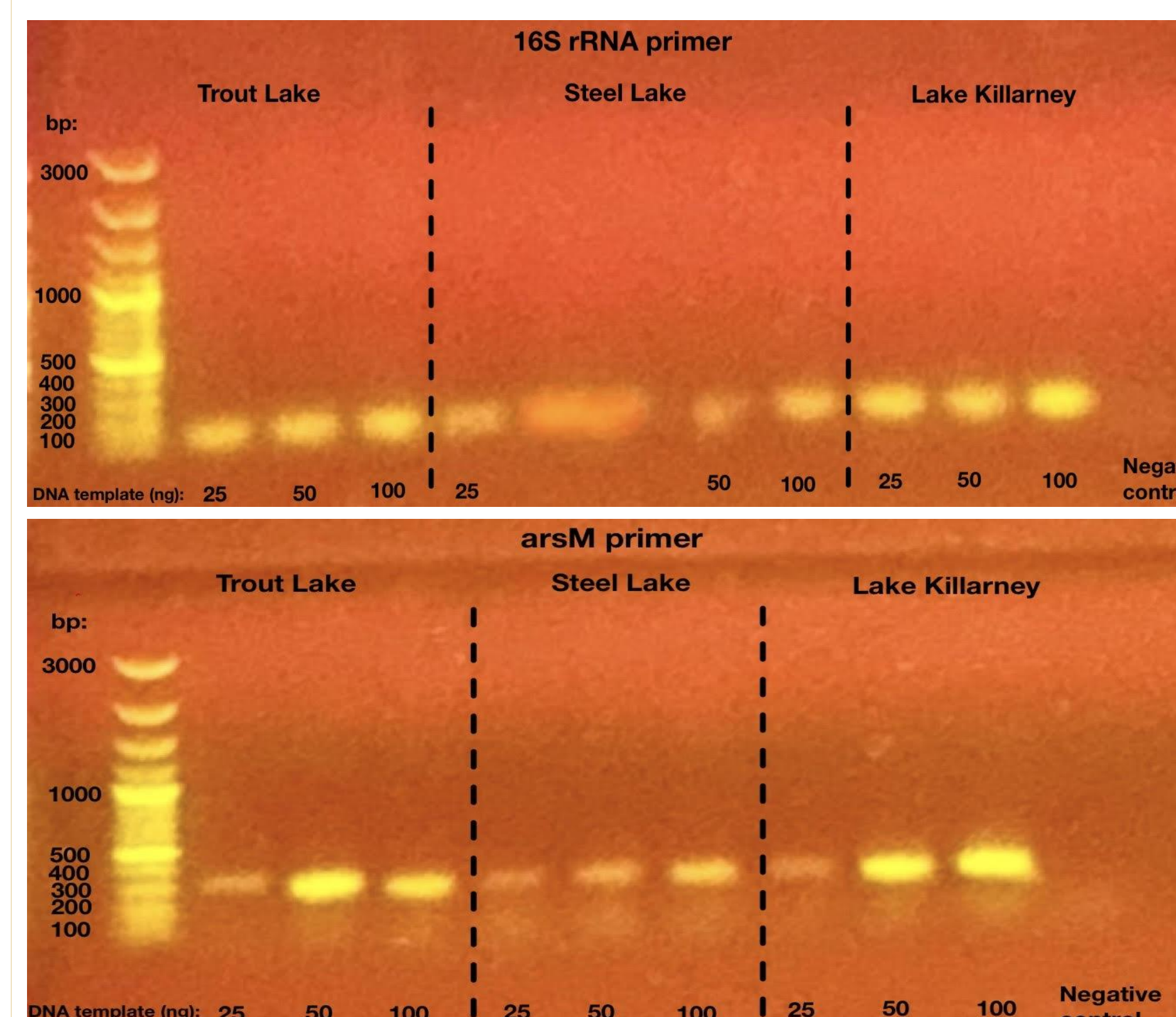### Arsenic Methyltransferase (ArsM) Gene Detected in Periphyton DNA

Figure 3: **Arsenite methyltransferase (ArsM) detected in periphyton DNA from all three lakes.** Periphyton DNA samples from Trout Lake, Steel Lake, and Lake Killarney were amplified at three concentrations using degenerate PCR and analyzed using agarose gel electrophoresis. Samples were paired with 16S rRNA or ArsM primers. Expected results for 16S rRNA (+ control) is around 100 bp while ArsM is expected around 300-400 bp. Indications for ArsM are present in each sample, with increased band intensity for samples containing higher concentrations of DNA. Negative controls show no sample contamination.

CONCLUSION

o Arsenic bioaccumulates differently across CMS tissues, with the highest concentrations observed in the gut of CMS from Lake Killarney.

o ArsM gene was present in all periphyton DNA samples from Trout Lake, Steel Lake, and Lake Killarney. This confirms that periphyton contain a gene that encodes for arsenite methylation into a less toxic form, which helps in its detoxification.

CONTINUED RESEARCH

- Repeating periphyton biotransformation analysis with different primers could improve results for other As biotransformation genes.

- Following this, reverse transcriptase quantitative PCR (RT-qPCR) could be used to determine levels of gene expression within the CMS. This may help us understand how host-gut microbiome interactions influence the biotransformation and metabolism of arsenic in CMS.

ACKNOWLEDGEMENTS

REFERENCES

Hull, E. A., Stiling, R. R., Barajas, M., Neumann, R. B., Olden, J. D., & Gawel, J. E. 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.

Nnaji ND, Onyeaka H, Miri T, Ugwa C. 2023. Bioaccumulation for heavy metal removal: a review. SN Applied Sciences. 5(5). https://doi.org/10.1007/s42452-023-05351-6.