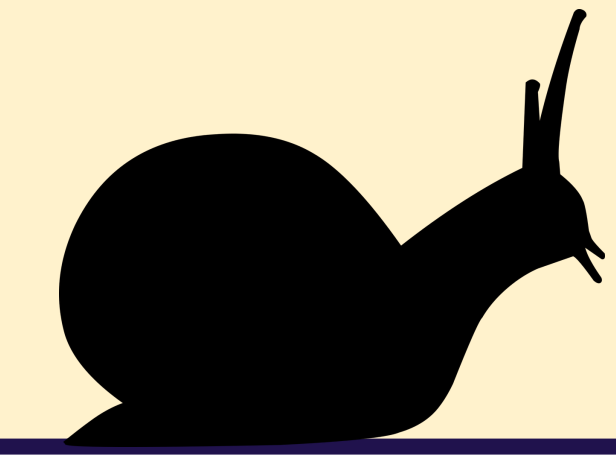


# Physiological Responses of Chinese Mystery Snails (*Cipangopaludina chinensis*) Following Arsenic Exposure

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## Abstract

The Chinese mystery snail (*Cipangopaludina chinensis*) is an invasive species that lives within the urban lakes contaminated with arsenic in south and central Puget Sound, WA. Chinese mystery snails can be exposed to arsenic from consumption of periphyton on lake vegetation, the water, and the littoral soil of the lakes they inhabit. This study evaluated a transcription factor of tumor necrosis factor alpha (TNF $\alpha$ ) as a potential biomarker for inflammation in the Chinese mystery snail. Following exposure to summer conditions when arsenic bioavailability is highest, vegetation and water were collected from three target lakes. The selected lakes in this study were Killarney (high arsenic), Steel (moderate arsenic) and Meridian (reference). After a two week period, snails exposed to lake vegetation and water were flash frozen and transcriptional regulation of LPS-induced TNF $\alpha$  transcription factor known as CcLITAF was assessed using reverse transcriptase PCR. Additionally, arsenic concentrations were measured in vegetation, water, and tissue using inductively coupled plasma mass spectrometry (ICP-MS). Total arsenic levels measured in plants and water from the three lakes matched the predicted trend. ICP-MS data revealed that though there was a greater amount of arsenic in Lake Killarney, the plant data for Lake Killarney was significantly higher than Steel and Meridian values. Current efforts are focused on further validation of TNF $\alpha$  transcription factor as a biomarker for arsenic exposure in the Chinese Mystery Snail.

## Introduction

- The Federal Way area was contaminated with arsenic from the ASARCO smelter which was located in Point Ruston area until 1993 (The History of Ruston way).
- The Chinese mystery snail is an invasive species in the Pacific Northwest which resides in the lakes contaminated with arsenic in the Federal Way area.
- Human health implications of consuming arsenic contaminated snails. Snails are used in Asian cuisine (Kingsbury et al 2021).
  - cLITAF could promote apoptosis in human tumor cell line (Yang et al 2012).
- Lipopolysaccharide (LPS) stimulates immune response for Tumor Necrosis Factor (TNF).
  - TNF is an acute inflammatory cytokine which is produced by macrophages and monocytes and promotes apoptosis, necrosis, and cellular signalling (Idriss et al 2000).
- Previous studies found arsenic concentrations for Steel at 1.75 ppb and Killarney at 20.42 ppb (Hull et al 2021).



Figure 1. Historical picture of ASARCO smelter located in Point Ruston, Washington. Photo courtesy of The History of Ruston Way source.

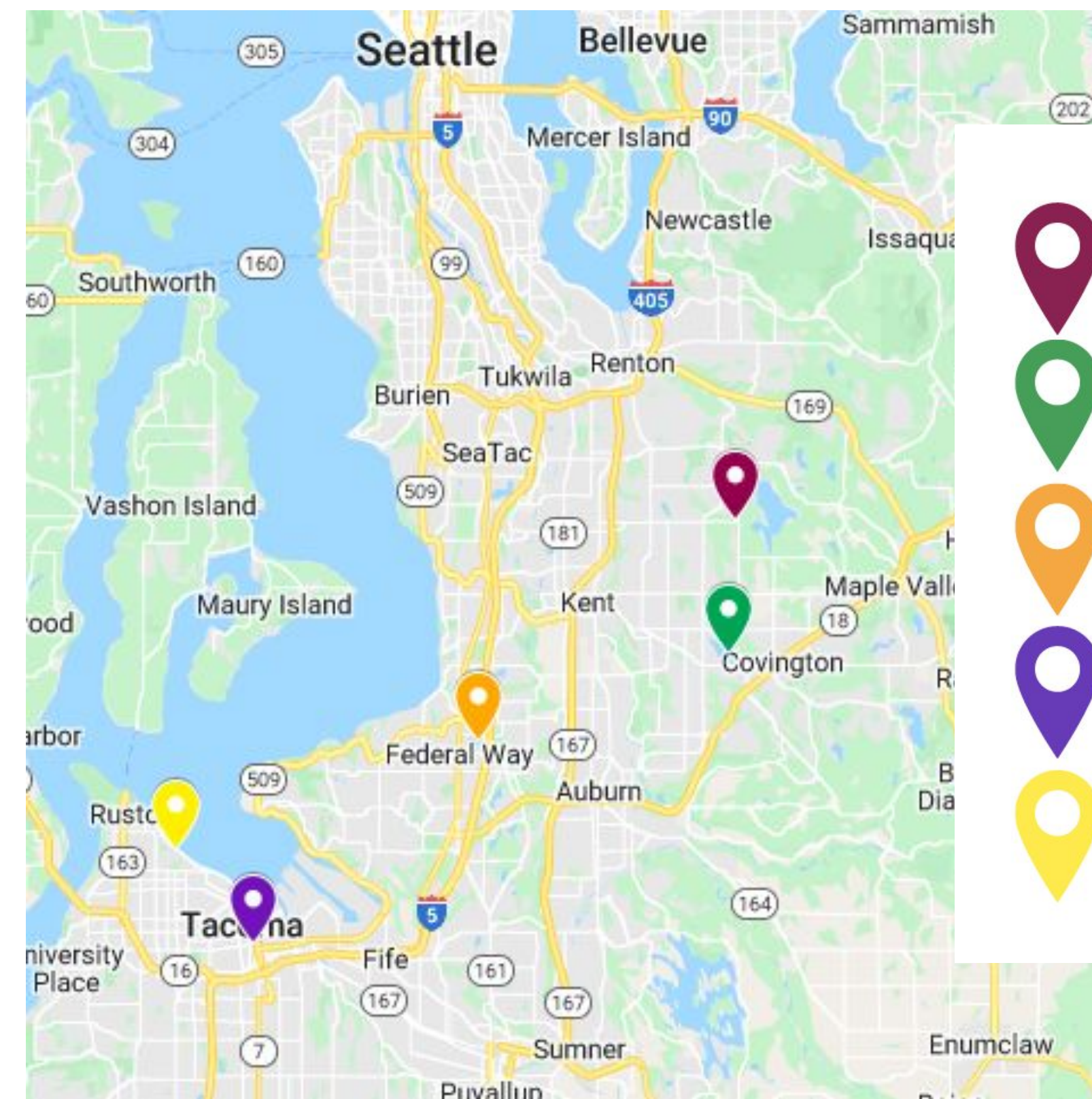


Figure 2. Map of relevant sites to study. Yellow indicates Ruston Way where the original ASARCO smelter began arsenic contamination. Purple indicates the University of Washington Tacoma where the research was conducted. Orange represents Steel Lake which is a moderate arsenic lake where vegetation and water were collected. Green represents Lake Meridian the reference lake of the study. Maroon indicates Lake Killarney, a high arsenic lake where water and vegetation were collected.

## Objective

Create a pilot study to expose snails from Lake Meridian to natural arsenic exposures from Lake Killarney and Lake Steel over a two week period. Dissect the snails into viable subsections and test the gut region for an LPS-induced TNF $\alpha$  transcription factor known as CcLITAF to determine inflammation factors of arsenic exposure.

## Results

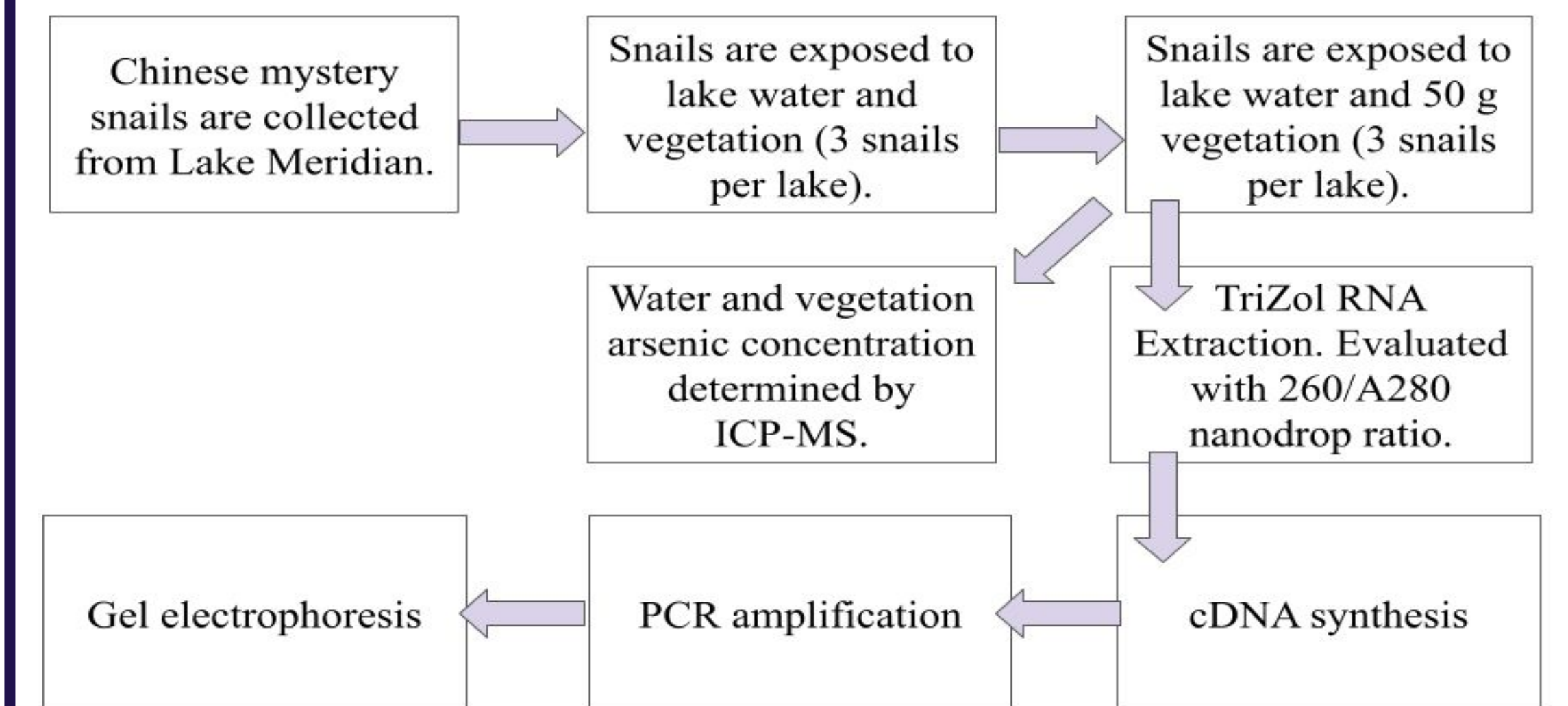
Lake Vegetation Sample	As [ppb]	As [ $\mu\text{g/g}$ ] dry wt
Steel	57.738	21.456
Steel	72.216	28.971
Steel	64.423	20.635
Killarney	666.392	298.517
Killarney	504.288	180.83
Killarney	589.717	248.029
Meridian	23.717	15.709
Meridian	2.0513	1.166
Meridian	8.367	3.3147

Table 1. ICP-MS data from water samples taken from unused water from pilot study. Lake Killarney has the highest concentration of arsenic measured with more than 5x the amount of arsenic than Steel and 62x the amount of arsenic as the control.

Lake Water Sample	As ( $\mu\text{g/L}$ )
Steel water	4.78
Killarney water	26.11
Control water	0.42

Table 2. ICP-MS data from vegetation samples taken from unused vegetation from pilot study. Arsenic values are highest for Lake Killarney and lowest for reference Lake Meridian. Wet and dry amounts are displayed.

## Methods



Primer Sequence (5'-3')	Gene Name
ACCTCCACCAAGCTACAGT	LITAF (Forward)
GATCAGACAACAGCCTAAC	LITAF (Reverse)
CACCGAATCCCTCATCCT	28SrDNA (Forward)
CTGCTCTGGACTGTCCTA	28SrDNA (Reverse)

## Discussion

- Lake Killarney arsenic concentration is significantly greater than Lake Meridian and Steel Lake samples.
- Lake Killarney water is also significantly greater than significantly greater amount of arsenic compared to control water and Steel Lake.
- Arsenic concentrations ( $\mu\text{g/L}$ ) were greater in the samples for this study compared to the samples from previous studies (Hull et al 2021).
  - Lake Steel 1.72 (Hull et al 2021) compared to 4.78.
  - Lake Killarney 20.42 (Hull et al 2021) compared to 26.11.
- Further studies are necessary to evaluate the use of TNF $\alpha$  transcription factor for arsenic toxicity in the Chinese mystery snail.

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