

# Physiological Impact of Freshwater Snails within the Puget Sound

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## Background

The ASARCO copper smelter operated for over 100 years in Tacoma, Washington contributing to heavy air pollution that settled on the surface soil and still remains until this day although the smelter has not been operational for over 30 years. The arsenic contamination of lakes surrounding the Puget Sound impose a threat to the species that inhabit them. Previous studies have shown the relevance of the gut microbiome on rocky mountain snails (*oreohelix strigosa*) to help with conservation and management of this species, but the relationship between heavy metal uptake and the gut microbiome is not well understood or established.

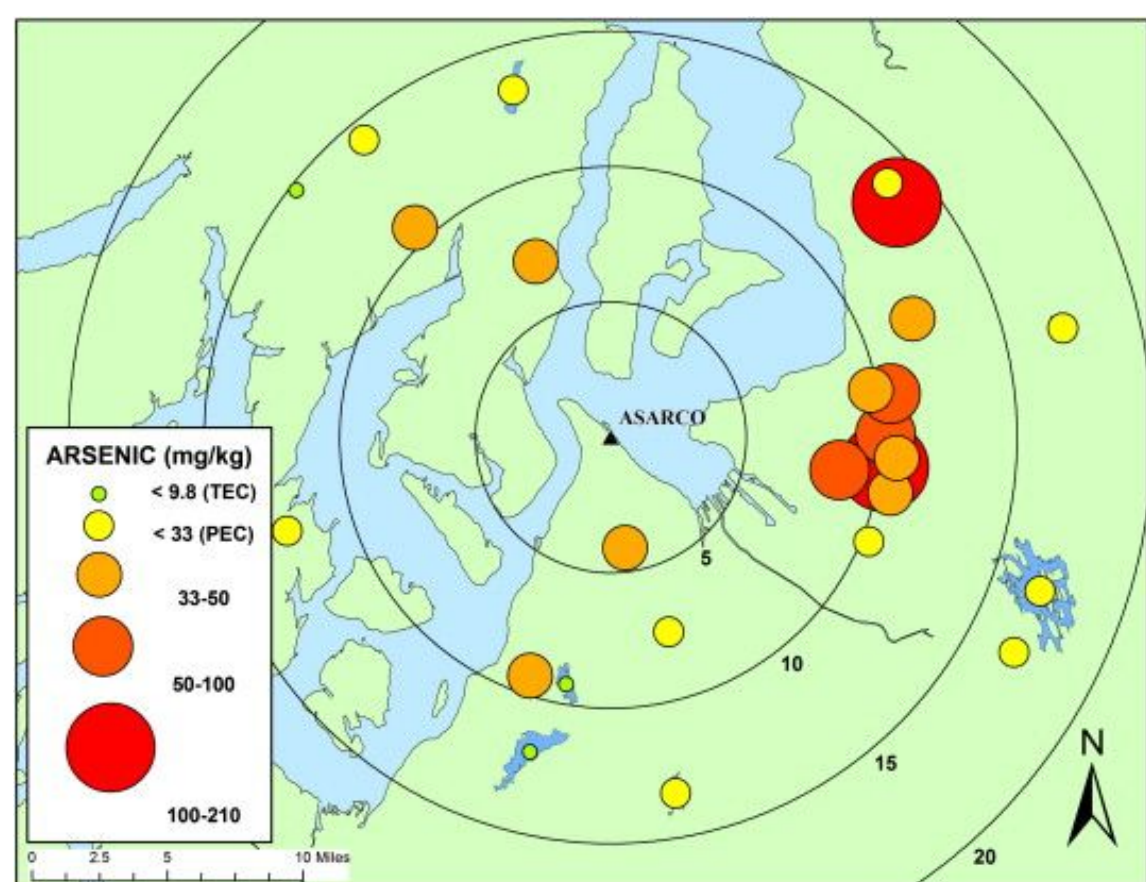


Fig 1. [As] (mg/kg) in surrounding lakes of the ASARCO smelter

HSP70 are a family of conserved ubiquitous heat shock proteins, high levels are produced by cells as a response to oxidative stress, hyperthermia, and changes in pH. **We hypothesize that there will be an increased HSP70 expression when there are more bioavailable arsenic sources to the organism.**

## Acknowledgements

Feeding exposures conducted by Dr. Allison Gardell and Summer Turnburg

## Reference

Gawel JE, Asplund JA, Burdick S, Miller M, Peterson SM, Tollefson A, Ziegler K, Arsenic and lead distribution and mobility in lake sediments in the south-central Puget Sound watershed: The long-term impact of a metal smelter in Ruston, Washington, USA, Science of The Total Environment, Volume 472, 2014, Pages 530-537.

## Methods

To test the heavy metal response, snails were fed plants in arsenic containing water from three lakes in the Puget Sound

- ☐ Killarney = High [As]
- ☐ Steel = Moderate [As]
- ☐ Meridian = Low [As]

After 2 weeks snails were crushed, dissected, and collected according to body part. BCA assays, SDS Page, and Western Blot were performed to detect HSP70 expressions.

## Conclusions

Our data showed that snails that consumed plants from the **least exposed arsenic** had a **higher expression of HSP70**. These results **do not support** our initial hypothesis that high arsenic levels will induce physiological stress in all snails.

## Future Directions

Continuing research could investigate the role of antibiotic resistance in the relationship between the microbiome, arsenic toxicity, and bioremediation. A more tractable experimental setup can include a more controlled environment of snails and exposure to Arsenic and antibiotics. Gastropods provide essential insights towards molluscan physiology and immunology (Allan and Blouin 2017).

## Results

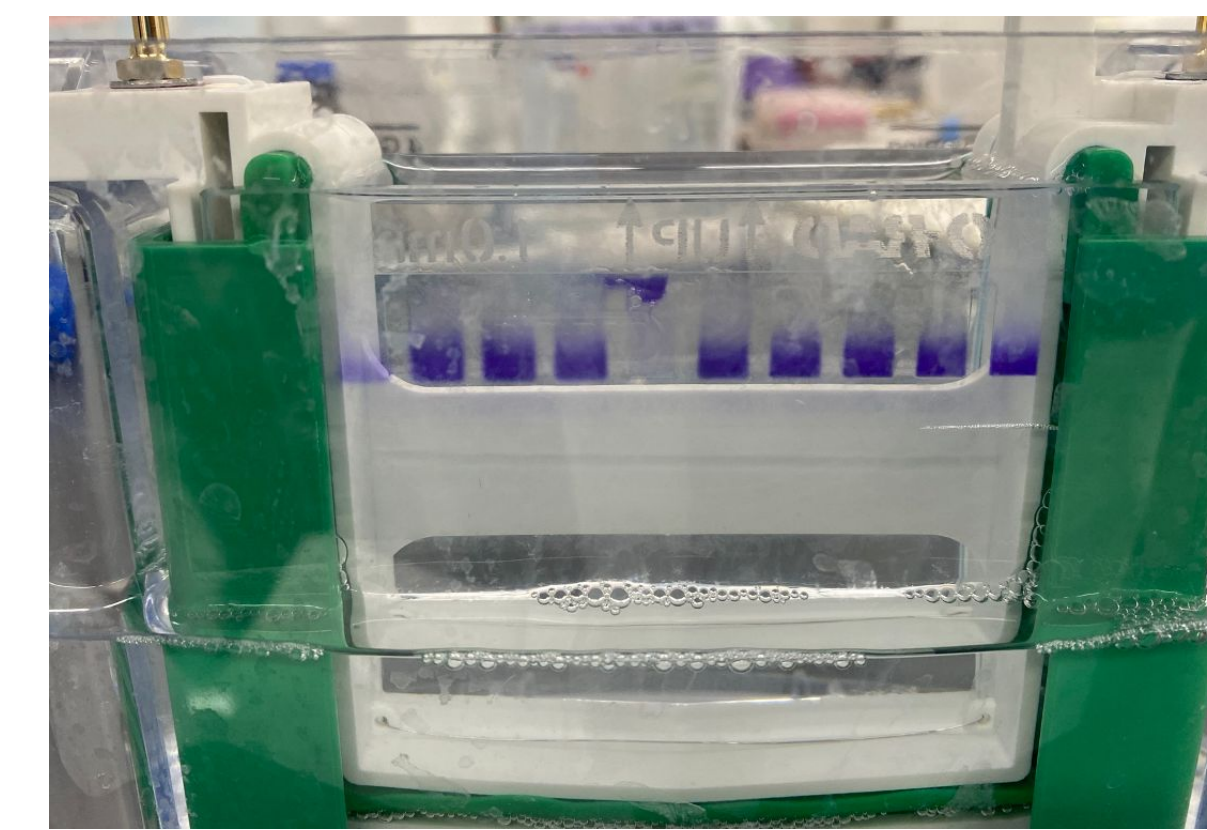


Fig 2. SDS-PAGE

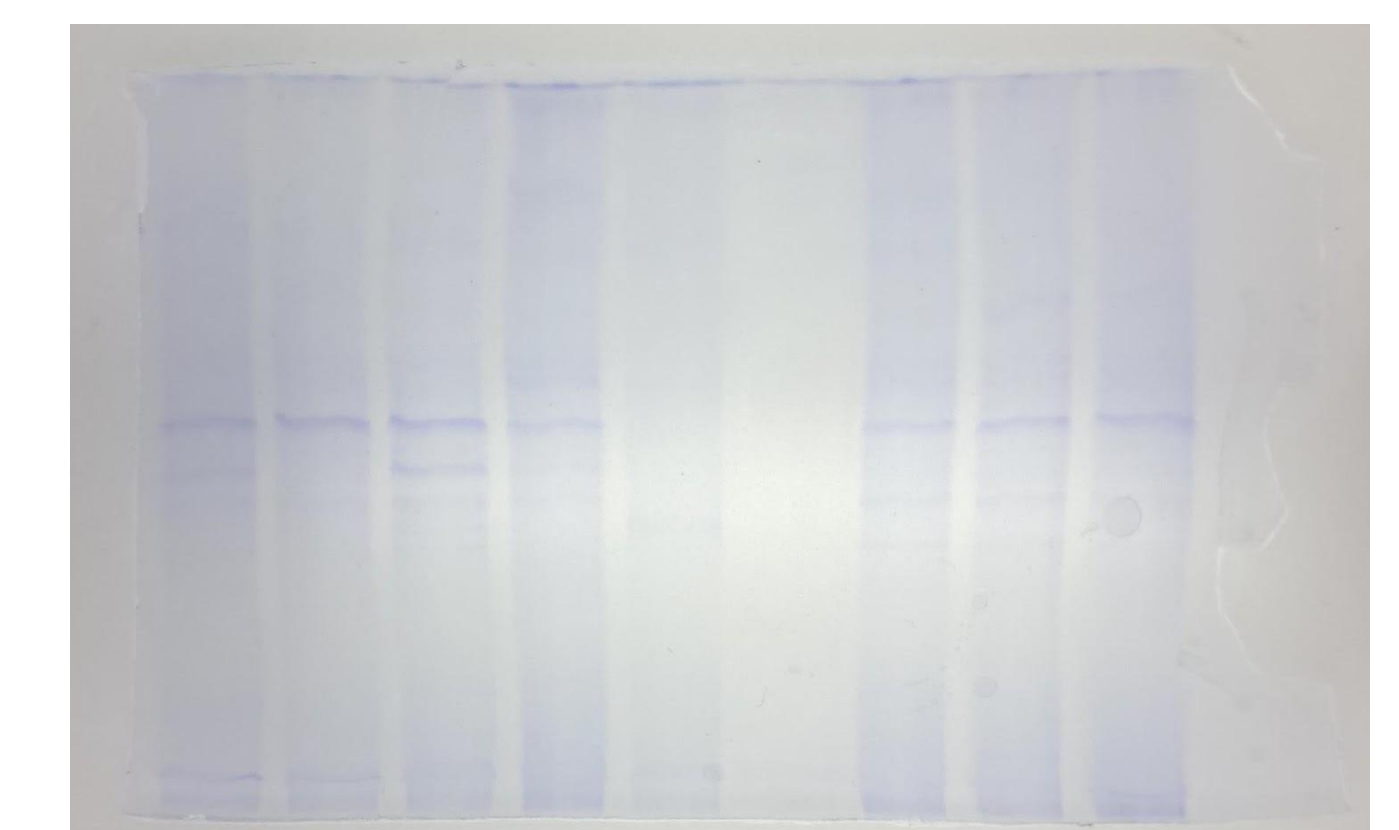
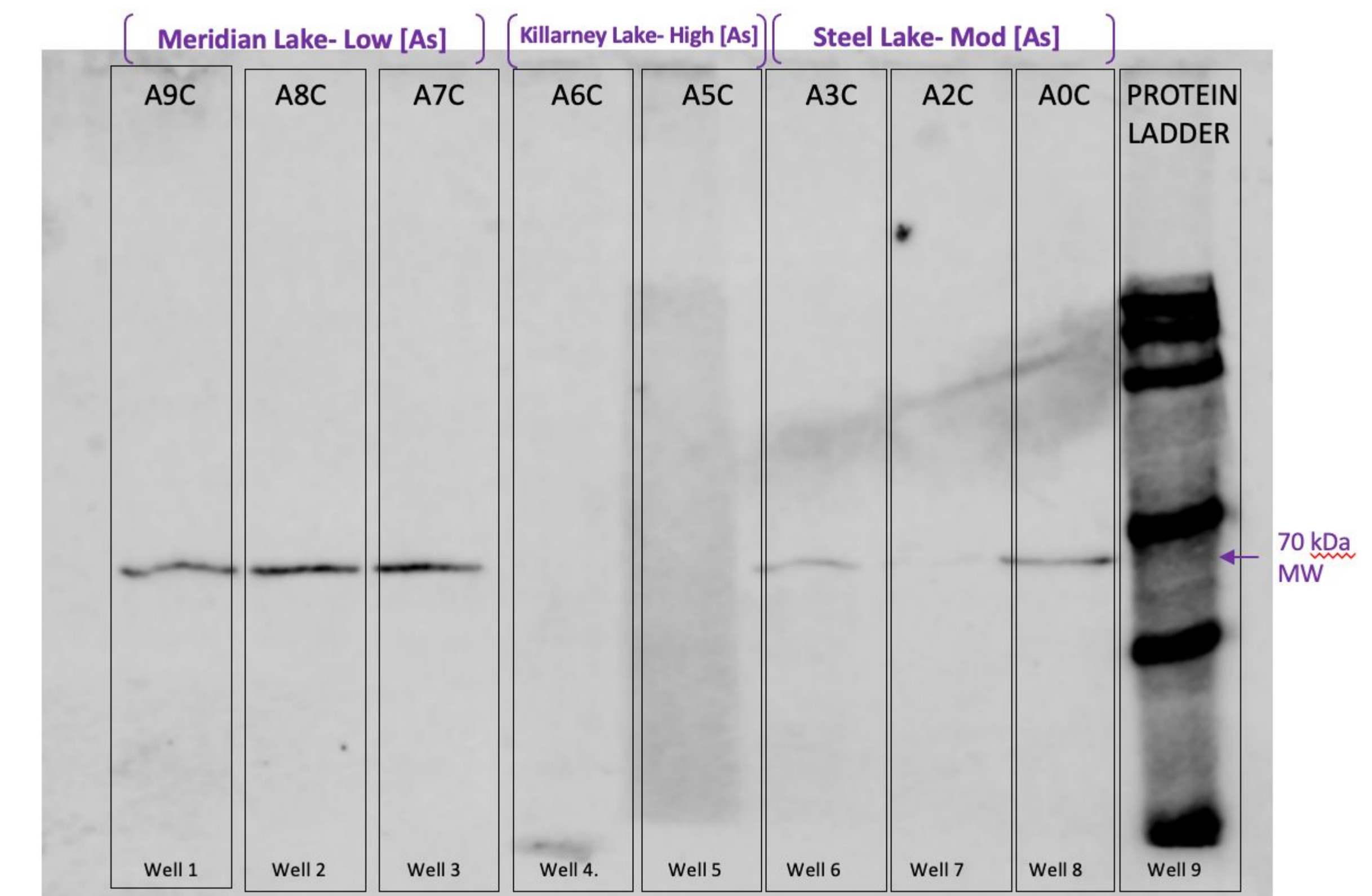


Fig 3. Coomassie Blue Stained Gel



## Figure 4. HSP70 Expression Western Blot

Physiological stress was quantified by probing HSP70 proteins from gut extracts from three different arsenic environments.

## Expression Summary

Plants Fed From ...	Relative HSP70 Expression Level (0,+,,+++)
Meridian Lake	+++ (head), + (gut)
Killarney Lake	+ (head)
Steel Lake	+++ (gut)