Examining the Links Between Physiological Stress and Microbiota in Snails Exposed to Environmental Toxins

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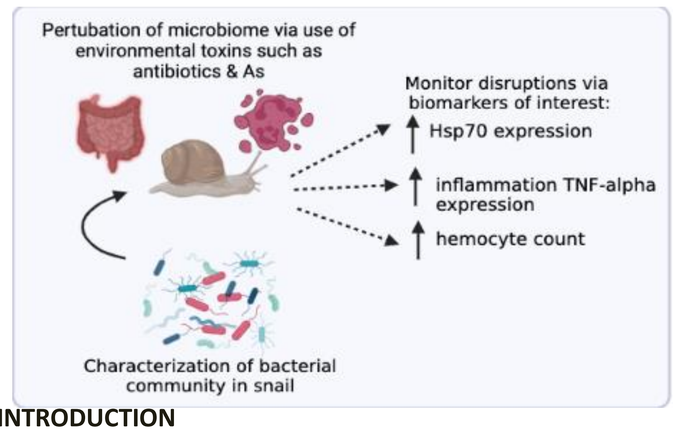


INTRODUCTION

Environmental toxins were predicted to disrupt animal microbiota. The impact of the microbiome disruption has been mechanistically linked to immune dysfunction in vertebrates[2,3]. However, the effects of environment toxins on the invertebrate microbiome structure and function have not been further explored. Arsenic and antibiotics are the two common environmental toxins used in this study. Arsenic toxicity is locally relevant due to arsenic emissions from the ASARCO copper smelter into freshwater lakes that contaminated the soil and water sediments around the Pierce and King county areas[1]. Antibiotics are the common effluents in wastewater including hospitals, research labs, and agriculture[2]. We used biomarker Hsp70 to detect whether physiological stress was induced in snails that may be linked to microbiome disruption. Hsp70 is a chaperone protein that catalyzes protein folding and degradation in response to a variety of physiological stressors[4].

Phase I Winter 2022: Wild-Caught *Cipangopaludina chinensis* snails extracted from Lake Meridian were fed with [As] exposed plants and periphyton from three lakes with distinct [As] to determine if physiological stress was induced and monitored via Hsp 70 expression.

Phase II Spring 2022-winter 2023: In response to the unexpected result from winter 2022 wild-caught snails, lab-reared snails ***Pomacea diffusa*** were exposed to antibiotics and Hsp70 expression was measured in a more tractable system.

HYPOTHESIS: If snails were exposed to environmental toxins such as arsenic or antibiotics, Hsp70 expression would increase due to an induction of their physiological stress response.

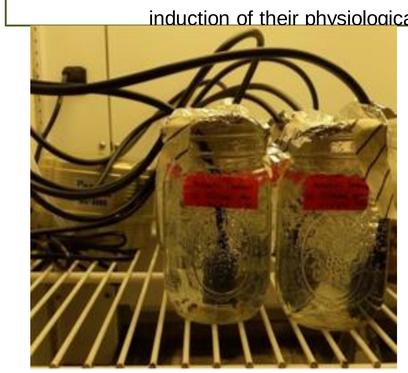


Figure 5. Antibiotic exposure of 6 juvenile snails per 16 oz jar were conducted in an environmental chamber with 12-hour light/dark cycles, 20C average ambient temperature and air bubbled into water via individual air stones.

RESULTS

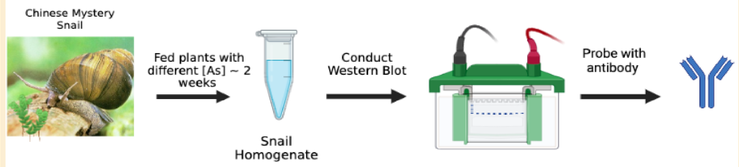


Figure 1: Chinese Mystery Snails extracted from Lake Meridian were fed plants from Lake Meridian (Low [As]), Steel Lake (Med. [As]) and Lake Killarney (High [As])

Figure 2: Western blot gel was completed to detect the expression of physiological stress protein, Hsp70, in wild caught *Cipangopaludina chinensis*.

Higher Hsp70 expression was seen in Lake Meridian fed snails which contradicted our initial hypothesis. This result introduced the possibility of setting up a lab-based experimental system where we could manipulate samples to determine the relationship between Hsp70 since it was unknown what factors influenced a result that contradicted our hypothesis.

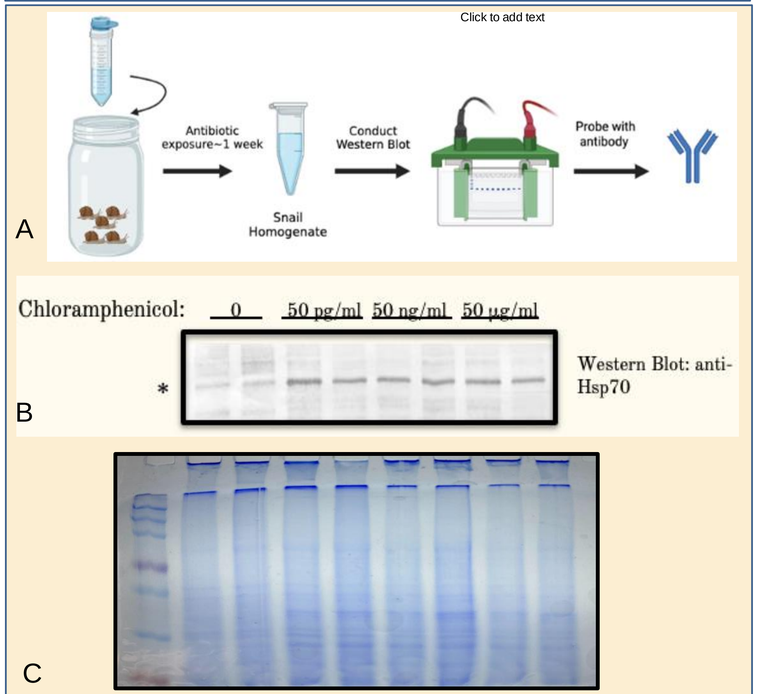


Figure 4. Hsp70 expression was induced in juvenile *Pomacea diffusa* snails via a one-week exposure to chloramphenicol (Clm). A) Snails were exposed to 0, 50 pg/ml, 50 ng/ml or 50 ug/ml Clm for 1 week to induce physiological stress and evaluate Hsp70 expression. After completion of the Western Blot, blot was probed with primary anti-mouse Hsp70 (1:1000) and secondary donkey-anti-mouse IgG alkaline phosphatase conjugate (1:1000). B) Hsp70 expression increased with exposure to all treatment concentrations of Clm. C) Coomassie blue stained gel confirmed equal loading of total protein in each lane of blot.

Antibiotic Sensitivity Tests

Research Goal: Test antibiotic sensitivity of the gut microbiome of the *Pomacea diffusa.*

One-month old snails were used for plate samples L1-L3 . Two-week old snails were used for plate samples S1-S3.

Figure 3A. Kirby-Bauer test indicated sensitivity of snail microbial communities exposed to 10 common antibiotics. Snail microbial communities showed a greater sensitivity towards tetracycline as indicated by the clear media surrounding this antibiotic disc whereas certain microbial communities were more resistant towards chloramphenicol.

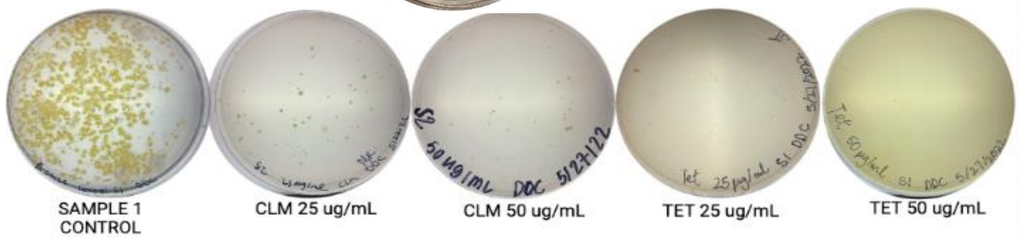


Figure 3B. Bacterial communities in lab-reared *Pomacea diffusa* showed a greater antibiotic sensitivity to tetracycline under both experimental treatments (25 ug/ml & 50 ug/ml) when compared to chloramphenicol (25 ug/ml & 50 ug/ml). Tetracycline caused the widespread disruption of bacterial communities found in *Pomacea diffusa* and would not be indicated for use in this experimental setup. Chloramphenicol disrupted the snail's microbiome as its concentration increased but did not completely sterilize all microbial communities.

CONCLUSIONS

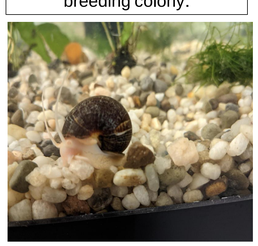
- Our initial experiment highlighted the need for a more tractable system where we can manipulate fewer variables at a time to effectively determine the relationship between environmental toxins, snail microbiome structure and physiological responses.
- We have preliminary data validating the use of Hsp70 expression as an indicator of altered interactions between bacteria and snail hosts.
- Microbiota of *Pomacea diffusa* are highly sensitive to tetracycline and sensitive to chloramphenicol.

FUTURE DIRECTIONS

- Determine if physiological stress is induced in lab reared *Pomacea diffusa* fed with arsenic-containing periphyton; correlate physiological stress with trophic transfer of As and change in microbiome.
- Develop an experimental cultivation system that promotes accumulation of As by periphyton.
- Determine if results of lab-based experiments persist when examining wild-caught snails from lakes with different As concentrations.

Frames and acrylic plates used to collect periphyton from local lakes/ Photo courtesy of the Gawel Lab, UWT.

An enthusiastic member of our newly established *Pomacea diffusa* breeding colony.



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ACKNOWLEDGEMENTS

We would like to thank Dr. Sarah Alaei and Dr. Alison Gardell for their contribution and mentorship and Melissa Ramirez Miralda, Dylan Culbert, Sahra Jama and the Winter TBIOMD 495 class for their contributions to this project.