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

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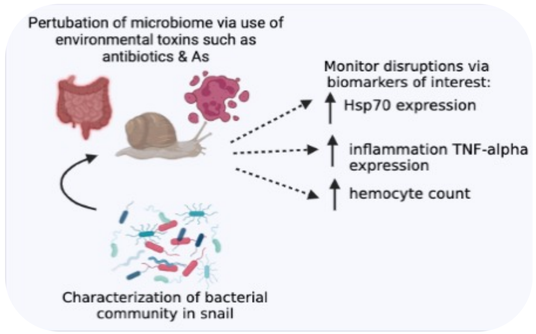
Apple Snail

Chinese Mystery Snail



INTRODUCTION

Environmental toxins were predicted to disrupt animal gut microbiota since disruptions of the microbiome have been mechanistically linked to immune dysfunctions in vertebrates[2,3]. However, the effects of environmental toxins on the structure and function of the invertebrate microbiome have not been fully explored. Arsenic and antibiotics are two commonly found environmental toxins that were used in this study based on their well-known ability to cause dysbiosis within vertebrates. 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 Pierce and King county areas[1]. Antibiotics are common effluents found in wastewater from hospitals, research labs, and agriculture[2]. In this study, Hsp70 proteins were used to determine whether a physiological stress response could be induced in snails, which may be linked to microbiome disruption. Hsp70 proteins are highly conserved chaperone proteins that participate in maintaining homeostasis, facilitate protein folding and protein degradation in response to a variety of physiological stressors[4]. Hsp70 proteins may be used effectively as biomarkers towards a wide-range of environmental toxins due to their role in metabolic processes and their quick response towards stressors.

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 a physiological stress response was induced and whether Hsp 70 expression was altered.

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

HYPOTHESIS: If snails are exposed to environmental toxins such as arsenic or antibiotics, then Hsp70 expression would increase due to stimulation 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, 20℃ average ambient temperature and air bubbled into water via individual air stones.

RESULTS

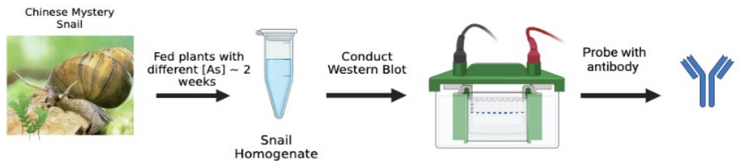


Figure 1: Chinese Mystery Snails extracted from Lake Meridian were fed plants from Lake Meridian (Low [As]), Steel Lake (Med. [As]) and Lake Killarney (High [As])

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

A higher Hsp70 expression was seen in snails fed from Lake Meridian [low As], which contradicted our initial hypothesis. It was determined that another factor other than arsenic may have contributed to this outcome. This result supported our reasoning for setting up a lab-based system in which we could manipulate individual variables to clarify whether Hsp70 expression was altered by environmental toxins.

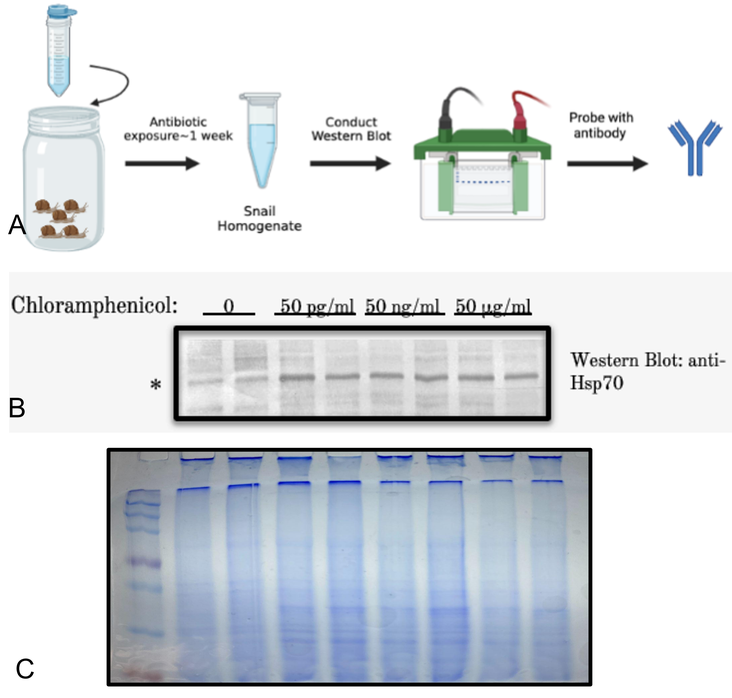


Figure 4. Hsp70 expression was induced in juvenile *Pomacea diffusa* snails via a one-week exposure to chloramphenicol (Clm). A) Snails were exposed to 0, 50 pg/ml, 50 ng/ml or 50 ug/ml Clm for 1 week to induce physiological stress and evaluate Hsp70 expression. After completion of the Western Blot, the 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 the total amount protein in each lane.

Antibiotic Sensitivity Tests

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

Apple snails were euthanized at two different time points, postnatal day 15 & day 30, to test the antibiotic sensitivity of their bacterial communities.

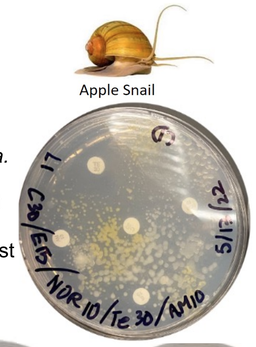


Figure 3A. Kirby-Bauer test indicated sensitivity of snail microbial communities exposed to 10 common antibiotics. Snail bacterial communities demonstrated a greater sensitivity towards tetracycline and less sensitivity towards chloramphenicol.

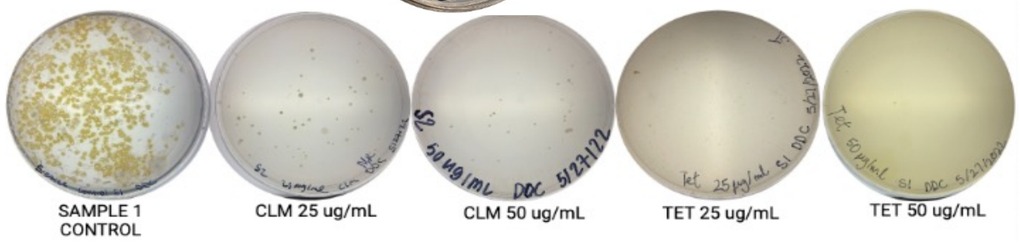


Figure 3B. Bacterial communities in lab-reared *Pomacea diffusa* showed a greater sensitivity towards tetracycline under both experimental treatments (25 ug/ml & 50 ug/ml) when compared to chloramphenicol (25 ug/ml & 50 ug/ml). Tetracycline caused the widespread disruption of bacterial communities found in *Pomacea diffusa* and would not be indicated for use in this experimental setup. The use of chloramphenicol was supported based on its ability to disrupt bacterial communities that did not cause widespread sterilization.

CONCLUSIONS

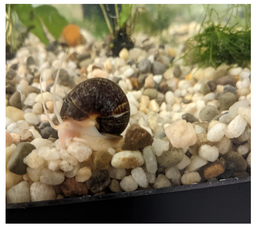
- Our initial experiment highlighted the need for a more tractable system where we could manipulate fewer variables at a time to effectively determine the relationship between environmental toxins, snail microbiome structure and physiological responses.
- We have preliminary data validating the use of 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 diiffusa* breeding colony.



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