

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

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The Asarco Company operated a copper smelter in Ruston, WA that generated significant amounts of heavy metal contaminants that settled on the soil and water sediments around the Puget Sound area. Our research was focused on understanding the physiological effects of environmental toxins in snails and how their microbiome was impacted by this exposure. Previous research noted high levels of inorganic arsenic in tissue samples of snails collected from Steel Lake and Lake Killarney located in King County, WA. In this research, snails were fed with plants from three different lakes that contain distinct arsenic concentrations (Meridian-Low [As], Steel- Mod [As], Killarney- high [As]) to determine whether a physiological stress response could be induced and whether elevated levels of arsenic would significantly impact Hsp70 expression. Results indicated that Hsp70 expression was induced in the head & gut tissue samples of snails fed with plants and associated microbes from Lake Meridian (low [As]) only. This result suggested that environmental factors other than arsenic exposure may have impacted the Hsp70 expression seen in this experiment. Thus, we shifted our focus to the development of a lab-based system using environmentally relevant toxins such as arsenic and antibiotics so we could manipulate one variable at a time. The microbiota of lab-reared snails was tested to determine its antibiotic sensitivity towards ten widely used antibiotics. Greater antibiotic sensitivity was noted in bacterial colonies exposed to tetracycline (30 ug) when compared to chloramphenicol (30 ug). Lab-reared snails were exposed to distinct concentrations of chloramphenicol (50 ug/ml, 50 ng/ml, 50 pg/ml) based on our objective to disrupt the snails' microbiota in a controlled environment and not cause its sterilization with a stronger antibiotic. Treated snails (~30 days of age) exposed to chloramphenicol were noted to induce Hsp70 expression at all antibiotic concentrations tested when compared to the control. Future research will consider assessing whether the Hsp70 results can be attributed solely to the disruption of the snails' microbiome or whether antibiotic toxicity may have contributed to the results seen.