Effect of Toxic Metals on the Marine Amphipod *Parhyale hawaiensis*

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Abstract

Background: Heavy metals are common industry-born pollutants that can cause a variety of mutations in aquatic life if their levels are left unchecked. The purpose of this study is to assess the use of a species of marine amphipod, *Parhyale hawaiensis* (Figure 1), as a biological indicator of heavy metal contamination.

Objectives: The research objectives were to quantify the lowest concentration of heavy metals at which the biological marker is effective, and to establish a relationship between concentration of heavy metal and expression of Glutathione-S-transferase (GST) in the arthropod.

Hypothesis: We predict that exposure of *P. hawaiensis* to high concentrations of heavy metals would result in increased GST gene expression. Additionally, we predict that the relationship between the concentration of heavy metal and the level of change in absorbance (representing gene expression) would be proportional, therefore as one increases, the other will too.

Methods: RT-qPCR was used to analyze the effect of incubating neonates of *P. hawaiensis* in various concentrations of heavy metal pollutants on the GST gene and 18S ribosomal RNA gene.

Conclusions: After trial exposures, it was concluded that diH_2O was not suitable as the medium due to high mortality of neonates (Figure 4). The optimal number of neonates for RNA extraction was determined to be 30 (Figure 3).

Results

Figure 1: *Adult P. hawaiensis*

Figure 2: *Tank setup for P. hawaiensis maintenance.*

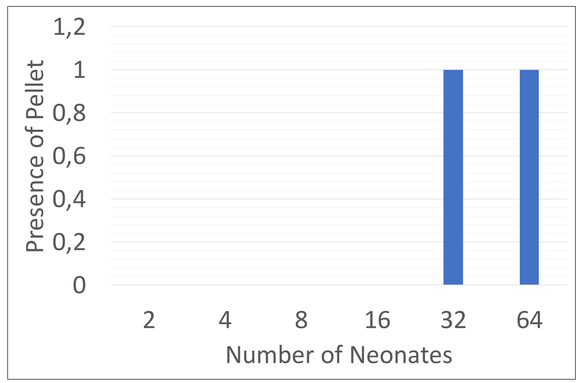

Figure 3: *Pellet presence based on number of neonates used in the extraction*

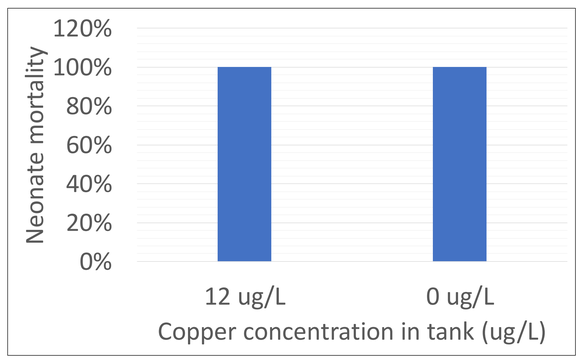

Figure 4: *Neonate death in positive and negative control exposures*

Expanded Methods

Maintenance of *Parhyale hawaiensis*

1. The cultures were separated into six tanks, each with at least four adults.
2. Each tank was cleaned and fed twice a week.
3. The salt level of the tanks was maintained at 1.025, calculated with a refractometer.
4. After each cleaning procedure, roughly 30 pellets of food were scattered throughout the tank.

Expanded Methods

Heavy Metal Exposure

1. Neonates were removed from the tanks and placed into two beakers with diH2O.
2. One had no copper metal added to it while the other had 20ppm.
3. Neonates were exposed for 8 hours, then transferred to a microcentrifuge tube.

Collection of *P. hawaiensis* and RNA extraction

1. 200uL of Trizol reagent were added to the centrifuge tube.
2. 0.2 mL of chloroform was added to the tube, then incubated for 2 to 3 minutes.
3. The sample was centrifuged for 15 minutes.
4. The transparent aqueous phase was transferred to a new tube.
5. 10uL of glycogen was added to the tube, followed by 0.5 mL of isopropanol.
6. The solution was incubated for 10 minutes, then centrifuged for 10 minutes.
7. A pipette was used to discard the supernatant, leaving an RNA pellet in the tube.
8. The pellet was washed with 1 mL of 75% ethanol.
9. The sample was then vortexed briefly and centrifuged for 5 minutes.
10. The supernatant was discarded. The RNA pellet was allowed to dry in air for 10 minutes.

Conclusion

The purpose of this project was to determine the optimal number of neonates that are necessary in order for an RNA pellet to formed. As shown in **Figure 3**, this number was determined to be 30, as numbers lower than that gave no pellet formation and numbers higher than that would be difficult to obtain often enough to perform the exposures in triplicate. As shown in **Figure** 4, incubation of *P. hawaiensis* with or without heavy metal exposure led to the death of the neonates being exposed, which led to the revision of the procedure .

Future exposures will be conducted in salt water instead of diH2O. Finally, the main achievement of the project was to establish a system of maintenance for the *P. hawaiensis* wherein they would be prompted to produce a large number of neonates per day (**Figure 2**). After many alterations in the original maintenance protocol, this was accomplished. The next step of this experiment is to re-conduct the heavy metal exposure experiment, and proceed from there.

References

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