Exposure to Tijuana River Estuary Sediments Decreased Heart Rate in Developing Medaka Embryos

Chaudhari A[1], Kwee M[1], Mahone V[2], McArthur W[3], Lu J[4], Vasquez M[4], Bozinovic G[4]

[1]Scripps Ranch High School, [2]Clairemont High School, [3]University City High School, [4]Boz Life Science Research and Teaching Institute

BOZ INSTITUTE

WHALE TAIL® LICENSE PLATE

Protect Our Coast and Oceans Fund

ABSTRACT

We examined the effects of pollutants in sediments on heart function and development of fish embryos, to assess potential effects of sediment exposure to fish present in the estuary. Medaka (*Oryzias latipes*) were used as model organisms in lab experiments due to their high tolerance to fluctuating salinities, defined development, and ease of maintenance. Our experiment found a significant relationship between sediment concentration and heart rate, with heart beats per minute decreasing as sediment elutriate concentration increased. The early exposure group (3 days) had a more significant drop in heart rate compared to the later group (7 days), suggesting higher sensitivity to the effects of pollutants in early development.

INTRODUCTION

Background

The Tijuana River Estuary (TRE), an estuary in southern California along the U.S.-Mexico border, is polluted by organic chemical pollutants from the surrounding urban areas, including polycyclic aromatic hydrocarbons (PAH) and phthalates[5]. Previous research has observed cardiac deformities in developing zebrafish embryos exposed to acute concentrations of these chemicals in isolation[6]. Heart physiology and development of aquatic organisms exposed to the polluted sediments in the TRE may also be adversely impacted. We propose to test these exposures using *Orizyas latipes*, also known as medaka, a model organism for studying estuarine environments due to its tolerance to a wide range of salinities.

Hypothesis

Increasing exposure concentrations of polluted TRE sediment elutriate will lead to a decrease in heart rate and a higher rates of bradycardia in medaka embryos.

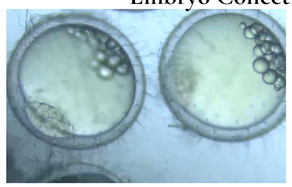
METHODOLOGY

Sediment Sampling and Elutriation

- Sediments samples were collected in the Tijuana River Estuary at 32°32'47.4900" N, -117°03'57.7476" W during dry season.
- Sediment elutriated following a modified EPA protocol (ref) using 15 ppt saltwater, mimicking brackish estuarine water.

Figure 1. Map of Tijuana River Estuary. Collection from sample site 2.

Embryo Collection & Exposure Setup



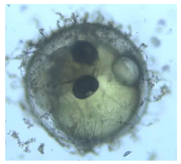
- Embryos are collected from the mother 1-2 hours after onset of light cycle in the fish system damaged embryos are removed.

Medaka embryos pre-exposure

- Embryos from the same mother divided evenly across exposure conditions to control for maternal influence.
- Aerated elutriate and 15 ppt saltwater before combining at 0%, 10%, 50%, 75% and 100% sediment elutriate
- 4 mL of each concentration, and pure 15ppt saltwater used as a negative control.

Embryo Microscopy

- Observed medaka embryos at 3 and 7 days post-fertilization (dpf).
- Determined developmental stage, measured heart rate, and recorded any observed deformities in development



Medaka embryo 7 days post-exposure

Data Analysis

- Analyzed and visualized data of medaka embryos' BPM (beat per minute) at 3 and 7 dpf using ggplot2 (4.0.1) in R (4.5.1)
- Shapiro-Wilk test used to determine if heart rate data normally distributed and Spearman Rank Correlation used to assess relationship between concentration of the elutriate and the BPM of the medaka embryos.

RESULTS

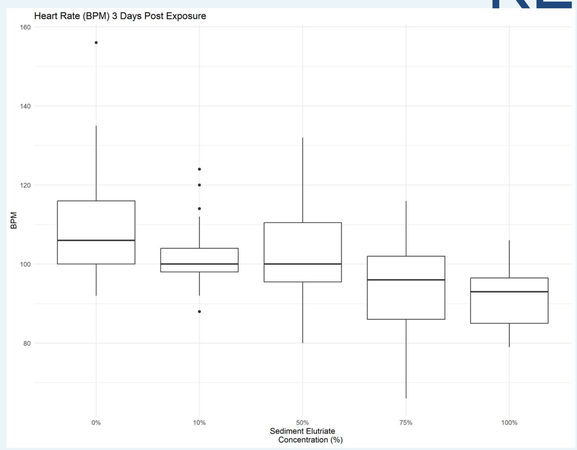


Figure 2. Heart BPM of the 3 dpf medaka embryos decreases as elutriate concentration increases. The Shapiro-Wilk test resulted in a p-value of 0.0002229. Spearman Rank Correlation test generated a rho value of rs=-0.4727433, suggesting a negative relationship between concentration of elutriate and BPM.

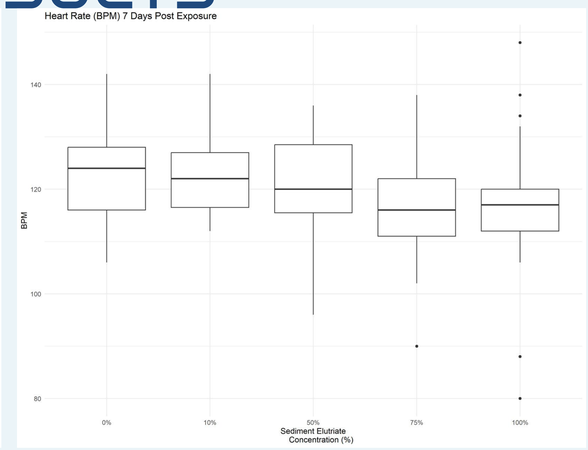


Figure 3. Heart BPM of the 7 dpf medaka embryos decreases as elutriate concentration increases. The Shapiro statistical test resulted in a p-value of $5.188*10^{-13}$. Spearman Rank Correlation test generated a rho value of rs=-0.2355515, a negative relationship between concentration of elutriate and BPM.

DISCUSSION

Conclusion

The results indicate a negative correlation between increasing concentration of sediment in the water and heart BPM of exposed medaka embryo, suggesting polluted TRE sediment elutriate can affect fish heart development. There was a stronger rho value for BPM observed at 3 dpf, indicating a greater impact on heart rate during early developmental stages compared to 7 dpf embryos. This may be an effect of the younger embryos under stress at critical rapid growth developmental stages where weight gain rates at these stages tend to be sigmoidal[4]. BPM data was also unlikely to be normally distributed from Shapiro-Wilk test, suggesting a real effect from sediment exposure. Hearts in early development are more susceptible to detrimental environmental conditions than fully looped and differentiated hearts at later stages[1] . Pollutants like PAHs have been shown to disrupt ion channels necessary for regulating the flow of K+ and Ca2+ ions, disrupting the electrical activity and conductivity of the heart and could lead to a slower heartbeat.[7] By 7 dpf, the cardiac system and relevant metabolic pathways of surviving embryos are more developed, suggesting a reduction in cardiac sensitivity to environmental pollutants.[1]

Limitations

1. Lab conditions cannot capture all TRE conditions: fluctuating temperature, salinity, and oxygen levels depending on the tides, precipitation, and season. While medaka can live in brackish water, they are not native to the TRE.
2. Microscopy limits: Low-power light microscopes limits detection of subtle tissue changes, molecular damage, and deeper internal deformities.

Future Directions

Observe and analyze brain and heart development exposed to increasing concentrations of sediments. To improve on the current experiment, we could test a larger sample size, sediments from more locations, use fish native to the TRE as a more representative model for developmental toxicity, and performed gene expression analysis on these fish to further analyze epigenetic alterations.

BIBLIOGRAPHY