No Significant Heart Rate or Differentiation Effect was Observed in Medaka Embryos Following Short-term Exposure to Tijuana River Estuary Sediment Elutriate

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ABSTRACT

The Tijuana River Estuary (TRE) is a coastal wetland historically polluted by sewage and wastewater. This research examines heart development of medaka (*Oryzias latipes*) fish embryos exposed to TRE sediment elutriate. While no significant difference was observed across exposure groups, further testing is needed to determine if there are long-term exposure effects.

INTRODUCTION

Hypothesis: Medaka embryos exposed to higher concentrations of Tijuana River Estuary sediment elutriate will experience greater heart developmental delays.

Tijuana River Estuary (TRE)

- Located along the southern border of the U.S., with freshwater inflows from both the U.S. and Mexico
- The estuarine water body is a mix of freshwater and seawater, as it connects the Tijuana River with the Pacific Ocean. Salinity levels vary across different locations.

Significance

- The TRE is the end point of Tijuana River where it enters the Pacific Ocean.
- An important stopover site for migrating birds.
- Polluted by nearby communities: industrial waste and sewage from Tijuana as well as pesticides from farms in the U.S.
- Pollutants detected in TRE include polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), which have been tied to both short and long term health effects, including cancer.[1,2]
- PCBs were banned in 1979, but are still detectable in TRE water.[2,3]

Figure 1. Representative photos of Tijuana River Estuary during high tide.

MATERIALS & METHODS

Sediment Collection

- Sediment sample was collected from TRE at an inland site (32.547251, –117.064224). The site is located on the main Tijuana River channel before it splits into distributaries.[3]

Sediment Elutriation

- Sediment elutriate was prepared according to EPA guidelines by mixing sediment and brackish water (15 ppt salinity) by 1:4 ratio.[4]
- The elutriate was further diluted to 10%, 50%, and 75% of the original concentration.

Medaka Exposure

- Medaka, a fish native to brackish habitats, were used as the model organism
- Four clutches of embryos were collected and exposed to diluted elutriate at Stage 9; brackish water was used for the control condition
- Developmental stages were determined based on established morphological criteria
- Heart differentiation was recorded at 3 days post exposure (dpe)
  - Scored on a 0-2 scale: 0 = not differentiated; 1 = beginning to differentiate; 2 = fully differentiated

Figure 2. Representative photo of an embryo with fully differentiated heart. The two chambers (atrium and ventricle) are indicated by arrows.

Data Analysis

- Heart rate was measured, and the data were found to be non-normal via Shapiro-Wilk test, and statistical significance across exposure groups was tested using Kruskal-Wallis test
- Data analyses were done in R and visualized using ggplot2 (v 3.5.1)

RESULTS

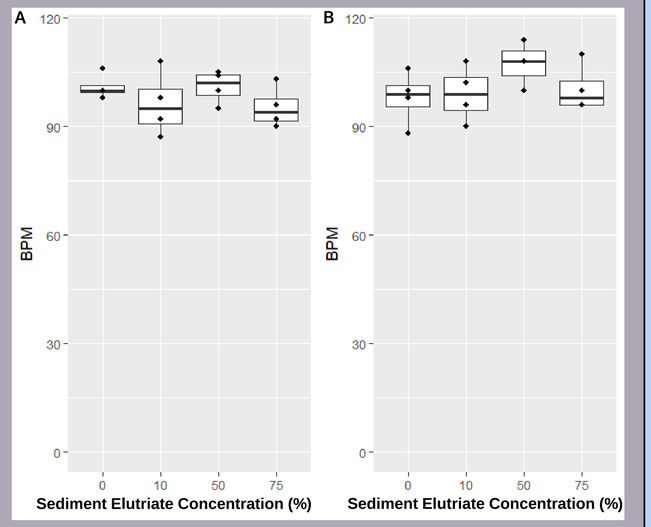

Figure 3. Heart beats per minute (BPM) of medaka embryos at (A) 3 days and (B) 7 days post exposure. There is no statistically significant differences across exposure groups at either time point ($p > 0.05$).

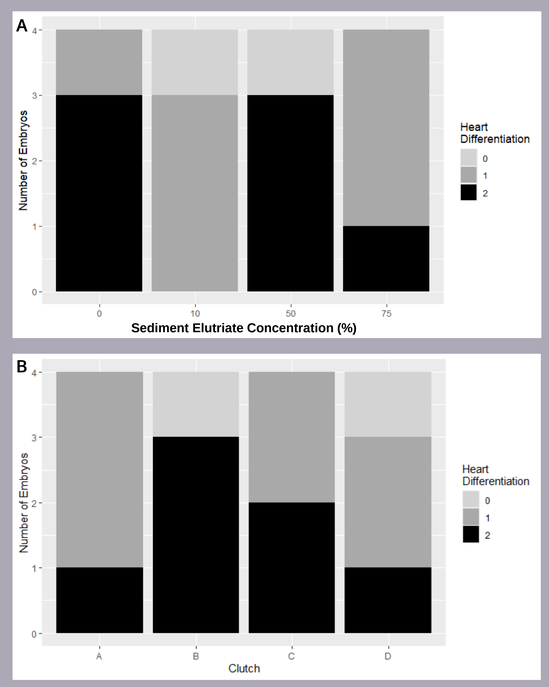

Figure 4. Heart differentiation progress at 3 dpe. There is no statistically significant difference in differentiation scores across (A) exposure groups or (B) embryo clutches ($p > 0.05$).

DISCUSSION

- Embryos exposed to 50% sediment elutriate had the highest mean heart rate in both groups (Figure 3). However, there are no statistically significant heart rate differences across exposure groups.
- At 3 dpe, the heart should be either close to or fully differentiated.[5]
- The control group has the highest mean differentiation score, while the 10% exposure group had the lowest score (Figure 4). Contrary to our hypothesis, the scores were not significantly different across groups.
- Delays in heart differentiation for other fish are shown to cause structural abnormalities as they grow older, affecting total lifespan.[6]
- We conclude that Tijuana River Estuary sediment elutriate did not affect heart differentiation or heart rate during early medaka embryo development.
- Variations across embryo clutches are comparable to variations across exposure groups. Batch effect from biological differences across mothers therefore did not significantly influence how the offsprings respond to TRE sediment elutriate.
- Further observation is needed to determine the long-term effects of early-stage delayed heart differentiation in adult medaka fish.

FUTURE DIRECTIONS

- Examine long-term and transgenerational effects of TRE pollution exposure on fish larvae and adults.
- RNA sequencing could detect more subtle exposure effects not observable at the physiological level.
- Salinity could also affect pollutant activities. Considering the salinity in TRE streams fluctuates depending on the weather, testing pollutant toxicity under various salinity levels can contribute to more accurate ecological assessment.

WORKS CITED