# POLLUTED SEDIMENTS FROM TIJUANA RIVER ESTUARY DELAY MEDAKA EMBRYO HEART DEVELOPMENT

Luu M.[1], Luu M.[1], Ryou A[2], Narenda N[3], Munaim N[4], Lu J[5], Feng Z[5], Bozinovic G[5]

[1]Mission Bay High School, [2]Rancho Bernardo High School, [3]Scripps Ranch High School, [4]East Village Middle College High School,

[5]Boz Life Science Research and Teaching Institute

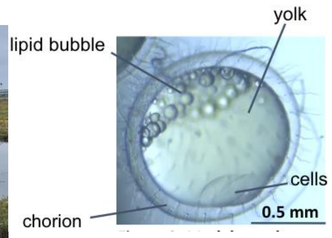
Research Question: Do toxicants from TRE sediment increase the likelihood of cardiac abnormalities in developing fish embryos, measured by heart differentiation ?

## Introduction

- The Tijuana River Estuary (TRE) is the home and breeding ground for a variety of fish and bird species. Anthropogenic pollutants in the TRE have had a significant impact on biodiversity in ecosystems and runoff from anthropogenic activities, such as agriculture and urban centers, traps pollutants in the estuarine sediments (Musegaas, 2024).
- Traces of PAHs, PCBs, and legacy pesticides such as EPTC and DDT have been detected in sediments samples from the TRE (Mclamb et al, 2023).
- The TRE is very biodiverse and a critical habitat for many aquatic organisms. Developing fish embryos are highly vulnerable to acute doses of pesticides and phthalates, leading to

  organ abnormalities (Renick et al, 2015)
- This study aims to investigate the effects of increasing dosages of chemical pollutants derived from TRE sediment on the heart development of medaka fish embryos. Medaka embryos are a suitable model for evaluating developmental toxicity effects on aquatic organisms because of their well-characterized developmental stages, their translucent embryos, and high tolerance to fluctuations in salinity.

**Tijuana River Estuary**




**Translucent medaka embryo, 2 hours post-fertilization**

## Hypothesis

**Research Hypothesis:** As the concentration of pollutants from TRE sediment to which medaka fish embryos are exposed increases, the likelihood of developing physiological abnormalities will also increase.

**Null Hypothesis:** No significant impact of concentration of TRE sediment elutriate on medaka fish embryo development

**Independent Variable**- Sediment Elutriate Concentration

**Dependent Variable**- Heart differentiation status at three and seven days post-fertilization

## Methodology

01 **Sediment Processing and Elutriation:**

- Sediment samples gathered from TRE, collected from site close to U.S-Mexico border (32.547251, -117.064224) and elutriated with 15 ppt saltwater following a modified EPA protocol

02 **Embryo Collection:**

- Lab-grown Medaka (*Oryzias latipes)* embryos collected from mother and exposed to sediment elutriate at 2-5 hours post-fertilization

03 **Dilutions:**

- Medaka embryos are exposed to different concentrations of the sediment elutriate, diluted with 15ppt saltwater at 1:10, 1:2, and 3:4 dilutions, along with pure 15 ppt saltwater and undiluted elutriate

04 **Observations:**

- Heart differentiation status of the embryos observed at three and seven days post fertilization via microscopy and recorded as fully differentiated, partially differentiated, or tubular

05 **Analysis:**

- Used R (4.4.3) software to conduct statistical analysis and the data is visualized utilizing ggplot2 (4.0.1)

## Results

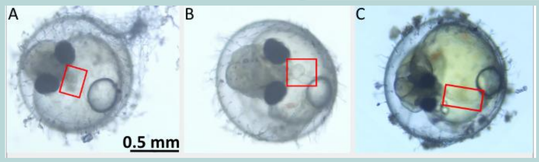



Figure 1. A) Fully differentiated, B) partially differentiated and C) tubular hearts in medaka embryos.

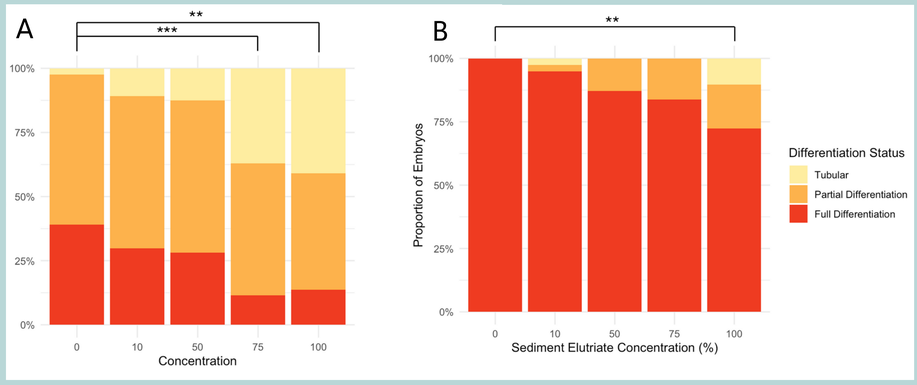



**Figure 2. General delay in heart differentiation as exposure concentration increases at both three days post-fertilization (A) and seven days post-fertilization (B).**

Statistically significant differences between groups visualized as **: p<0.01 & ***: p<0.001.

## Data Analysis

- Shapiro-wilk tests determined that the distribution of data was nonnormal ($p<0.05$) for both timepoints
- Kruskal-wallis tests ($p<0.05$) followed by Dunn's tests with Bonferroni-Holm corrections were performed on both datasets

## Discussion and Future Directions

- As the elutriate concentration increases, the proportion of embryos with tubular or partially differentiated hearts increased (Fig 2). Our hypothesis was supported by our data, indicating higher concentrations of sediment elutriate also increases the likelihood of cardiac abnormalities and delays in heart differentiation.
- Analysis between sediment concentration and heart differentiation stage showed significant differences in both 3dpf and 7dpf between 100% elutriate and the control group. At 3dpf, there was also a significant delay in development in the 75% elutriate exposure compared to the control group.
- PAHs, PCBs, and endocrine disruptors such as phthalates are associated with adverse effects on heart development at acute doses (Holme et al., 2019; Teixido et al., 2019; Wang et al., 2021). These chemical classes have been detected in this estuary (McLamb et al., 2023) and our study suggests the exposure to these complex chemical mixtures could have similar adverse effects on developing aquatic organisms within the estuary.

### Limitations

- The observed morphological effects cannot be attributed to any specific chemical in this complex mixture
- The bioavailability of chemicals identified in the sediment across the embryo chorion is unknown

### Future Directions

- Correlate morphological effects with differential gene expression analysis
- Identify the body burden of exposed medaka embryos and the chemical composition of the sediment elutriate
- Utilize more complex models to study embryo morphology

Bibliography