Exposure to Polluted Sediment Elutriate from the Tijuana River Estuary Results in Developmental Delay and Deformities in Medaka Embryos

Lin, N.[1], Yao, F.[1], Yao, J.[1], Jiang, S.[1], Lu, J.[2], Vasquez, M.[2], Bozinovic, G.[2]

[1]University City High School, [2]Boz Life Science Research and Teaching Institute

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Abstract

The Tijuana River Estuary (TRE) is a site of historical environmental deprecation, with human encroachment dating back to the late 19th century. As a result, a variety of pollutants are now present in the area, posing great risks for both the variety of people and the wildlife that call the area home. We have exposed medaka fish (*Oryzias latipes*) embryos to varying levels of polluted sediment elutriate concentrations in order to gauge the effects on fish development. We observed that developmental delays were positively correlated with elutriate concentrations, highlighting the polluted sediment's potential risk for aquatic organisms in the TRE.

Introduction

Research Question:
How do the environmental toxicants in the Tijuana River Estuary affect the development of fish?

Hypothesis:
Exposure to polluted sediments from the Tijuana River Estuary will delay development of the organ systems in medaka embryos

Tijuana River Estuary

- Located at the U.S.–Mexico border
- Historically polluted by urban runoff, industrial and agricultural wastewater, and sewage spills, especially at inland, upstream regions
- Toxicants found in sediments from the estuary include neuro- and immunotoxic pollutants such as polycyclic aromatic hydrocarbon (PAHs) and phthalates (McLamb et al., 2024)

Medaka

- Used for embryotoxicity test to assess the ecological risk of the TRE by observing embryo development after exposure to elutriated sediment containing environmental toxicants
- Great model organism due to their quick development period, translucent embryos, hardiness, and tolerance to a wide range of salinity

References

Methodology & Study Limitaions

Sediment elutriation

- Sediment sample collected from an upstream site in the TRE (32.547251, -117.064224), characterized as high in PAHs, polychlorinated biphenyls (PCBs), pesticides and phthalates, was used
- Sediment elutriate was prepared with 15 ppt saltwater at a 1:1 ratio following 30 minutes of mixing and 1 hour of settling.

Embryo Exposure

- 215 embryos were exposed to different concentrations of the sediment elutriate diluted with 15 ppt water (0%, 10%, 50%, 75%, and 100%) for 7 days +/- 1 hour
- All embryos from clutches with dead embryos in the control group were excluded from analyses

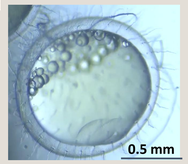
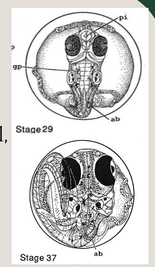


Figure 1. Medaka embryo prior to exposure, two hours post-fertilization.

Data Collection & Analysis

- Embryo morphology were assessed through microscopy around 3 days and 7 days of exposure to determine survival, developmental stage, heart differentiation, and heart rate
- Developmental stage was visualized with a linear regression line of best fit across elutriate concentrations



Expected stages at 3 and 7dpf (Iwamatsu, 2004)

Study Limitations

- We were unable to pinpoint the individual effects of the toxicants in sediment elutriate on embryo development
- Staging is based on a few morphological endpoints observed through microscopy
- Body burden of the medaka embryos is unknown

Results

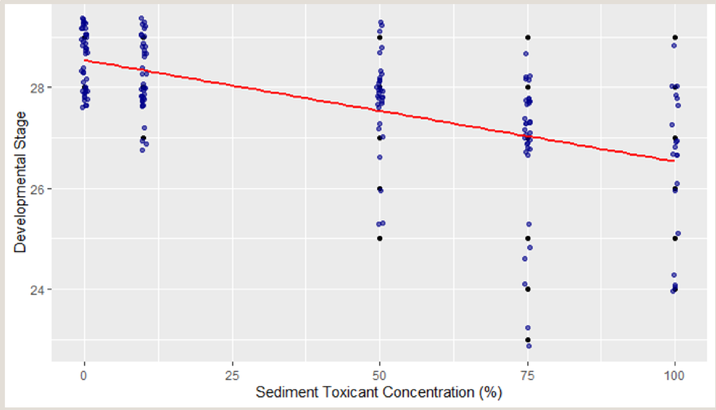


Figure 2. Linear regression (red line) highlights significant negative correlation ($p < 0.05$) between medaka embryos developmental stage and elutriate concentration at 3 days post fertilization. R^2 = 0.3394349

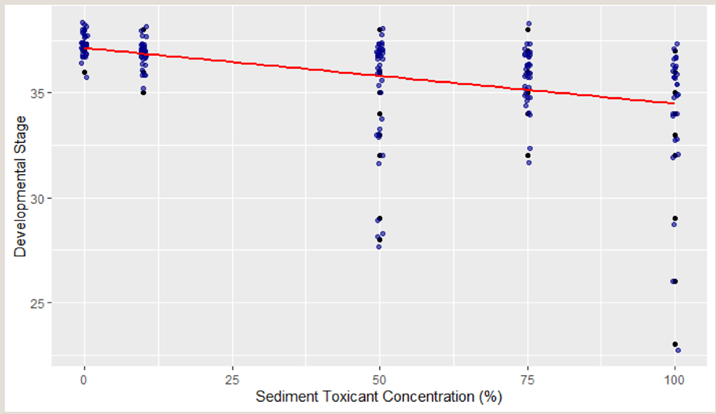
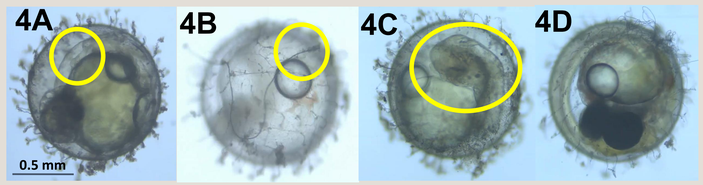


Figure 3. Linear regression (red line) highlights significant negative correlation ($p < 0.05$) between medaka embryos developmental stage and elutriate concentration at 7 days post fertilization. R^2 = 0.1934418



Figures 4. Medaka Embryos with highlighted deformities: embryos with tubular heart (A), hemorrhage (B), and lack of eyes (C). Control embryo from 0% elutriate solution is shown in D.

Discussion

- Developmental stage is negative correlated with elutriate concentration, both early in embryonic development (Fig. 2) and later, after 7 days of development (Fig. 3)
- Observations showed hemorrhaging of the body in higher sediment elutriate concentrations and delays in heart differentiation (Fig. 4).
- These issues may be associated with the two classes of organic pollutants found to have the highest abundance in the TRE sediments (McLamb et al., 2024): polycyclic aromatic hydrocarbons (PAHs) and phthalates.
- PAHs have been shown to cause edema, hemorrhaging, and cardiomyopathy, in many fish species such as herring and ricefish (Ha et al., 2019, Incardona et al., 2023).
- Hemorrhaging and undifferentiated hearts in our exposed embryos could also have been caused by PAHs in the polluted sediment elutriate
- Phthalates, a group of plasticizers, are also associated with adverse developmental effects (Kwan et al., 2021) and can damage neurons in the cholinergic system (Liu et al., 2025) that produce acetylcholine.
- Damage to acetylcholine leads to further developmental issues, as Younes, 2023 identified acetylcholine as an essential component for spontaneous neural activity, and Brennan et al., 2005's found that acetylcholine is necessary for proper muscle development.
- Phthalate exposures have also been associated with epithelial cell damage, inflammation, and hemorrhaging
- The exposed embryos' body burden of pollutants should be further quantified to help further determine if PAHs and phthalates are the only contributors to the adverse effects.

Conclusions + Future Directions

The Tijuana River Estuary, despite its immense ecological value, have been heavily polluted. Our data contributes to the growing evidence that toxic chemicals leaking into the environment could harm the wildlife. Observed exposure effects during medaka embryo development, including hemorrhaging and developmental stunting, could have been caused by two groups of toxicants with relatively high abundance in the TRE, PAHs and phthalates. While the exact chemicals driving the toxicity are yet to be determined, urgent protection and cleanup of the TRE are warranted to prevent further damage. Future studies should identify individual and combined toxicological effects of TRE contaminants. Monitoring efforts will also help assess the variations in toxicant abundance in TRE.