Tijuana River Estuary Sediment Exposure did not Induce Significant Effects on Developmental Rate or Morphology in Medaka Embryos

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Abstract

The Tijuana River Estuary (TRE) has been historically polluted by urban runoff, industrial wastewater, and transboundary sewage spills that threatening local ecosystems. Developmental toxicity was tested using *Oryzias latipes* (medaka) embryos with sediment samples collected from the TRE during a dry season. At two critical developmental stages, embryos were assessed for developmental delays and morphological deformities in response to TRE toxicants. Our data revealed no significant effect in developmental rate or hemorrhaging across sediment elutriate concentrations. Further tests are needed to more comprehensively assess possibly affected biological processes.

Introduction

Hypothesis

- Developmental delays and hemorrhaging occurrences will be greater in embryos exposed to higher concentrations of sediment elutriate

Tijuana River Estuary (TRE)

- The TRE, California's largest coastal wetland, lies on the Mexico-U.S. border
- It is a critical ecological habitat supporting hundreds of bird and species[1]
- Fertilizers, organic pollutants, and industrial waste flow into the estuary and can threaten ecological[2] and public health

***O. latipes* Embryo Development[3]**

- Medaka embryonic development is segmented into 39 stages based on morphologic diagnostic features
- On average, medaka embryo development takes 9 days from fertilization to hatching
- Stages 29 and 37 are critical stages to embryo development and thus selected for observation. Deviations from the expected diagnostic features at these critical stages, such as heart deformities or hemorrhaging, serve as indicators of sediment toxicity
- Stage 29 on average occurs 3 days post-fertilization (dpf). It is marked by heart differentiation, a feature crucial for proper circulation
- Stage 37 on average occurs 7 dpf. By this point, the pericardial cavity has developed and should be easily observed

Figure 1. Sediment sampling site at the Tijuana River Estuary. As part of a larger scale study, the sample used in this study was collected from the site highlighted in red.

Materials and Methods

Sample Collection

- Sediment was collected from the Tijuana River Estuary at 32.547251, -117.064224
- Samples were collected during a dry season on 12/12/2020

Sediment Elutriate Preparation

- Elutriate was prepared by mixing the sediment with brackish water (15 ppt salinity) in a 1:4 ratio[4]
- The same brackish water was also used for the control condition and for preparing diluted elutriate

Medaka Exposure and Observation

- *O. latipes* embryos at developmental stage 9 were exposed to 7.5 mL of the four elutriate concentration (0%, 50%, 75%, and 100%) and incubated at 25˚C. (n = 4 per condition)
- Embryos were staged at 3 and 7 dpf and evaluated for morphological deformities and hemorrhaging

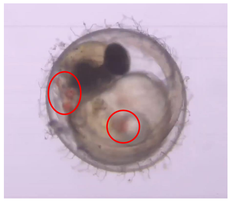

Figure 2. Representative photo of a hemorrhaging embryo. Two bleeding areas (head and tail) are highlighted by circles.

Data Analysis

- One-way ANOVA ($p<0.05$) with Tukey's post-hoc tested for significant developmental stage differences across elutriate concentration exposures
- Hemorrhage data were not normal via Shapiro-Wilk test ($p<0.05$). Significance was tested using the Kruskal-Wallis test. All analyses were done in R. Data was visualized using ggplot2 (v 3.5.1).

Results

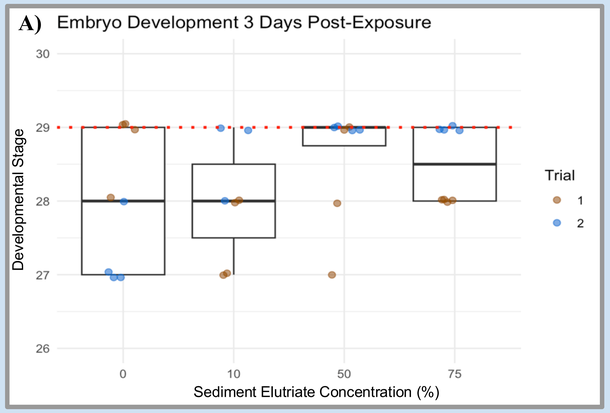

Figure 3. Developmental stages of *O. latipes* embryos at (A) 3 and (B) 7 days dpf across exposure conditions. The dotted red line represents the expected stage at the respective timepoints. Boxes indicate medians and quartiles of total data. Trials 1 (n = 16) and 2 (n = 16) are color-coded brown and blue. There were no significant developmental stage differences across elutriate concentrations ($p > 0.05$).

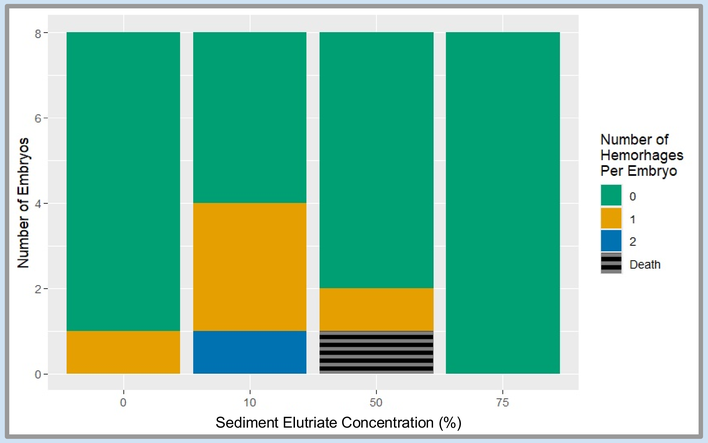

Figure 4. Most embryos are normally developed by 7 dpf in all experimental groups. Each bar represents a sediment elutriate concentration, and each color represents the number of hemorrhage spots within an embryo. There were no significant differences across elutriate concentrations ($p > 0.05$).

Discussion & Conclusions

- Fish embryos in earlier stages of development are particularly sensitive to water pollution. Early-stage chorions are not developed enough to protect them from pollutants, especially heavy metals, which can result in developmental toxicity and influence developmental rate.[6]
- Developmental stage varied more at 3 dpf relative to 7 dpf (Figure 2).
  - There are 6-10 hours expected per developmental stage from 27 to 29, and 12-24 hours per stage from 35 to 37.[5]
  - The longer stage durations may be have resulted in the lower stage variations in the 7 dpf groups.
- Developmental stage distribution at 3 days post-exposure varied between the two trials (Figure 2A).
  - More embryos in the control group were at the expected stage 29 in trial 1
  - In trial 2, more embryos in the two high exposure concentration groups were at stage 29.
- Contrary to our hypothesis, higher pollutant exposure levels did not result in more delayed embryo development or more abnormal development.
- Nevertheless, it is possible that other biological processes can be affected by the TRE pollutants, and further studies are needed for more comprehensive assessments.

Study Limitations and Future Directions

- The sediment elutriate prepared only represented a single dry season sampling event.
  - long-term monitoring with frequent sampling could capture the temporal fluctuations in the sediment chemical composition
- Only one species was used for toxicity testing
  - testing more aquatic organisms, including invertebrates such as snails and clams commonly found in TRE, should help assess the ecological impact of pollution more accurately

Works Cited