Tijuana River Estuary Sediment Elutriate Exposure Results in Elevated Mortality and Slowed Heart Differentiation During Medaka Embryo Development

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Summary

- Tijuana River Estuary sediment was assessed for developmental toxicity potential with thirteen medaka fish embryo groups (n = 56) from six mothers.
- Embryos exposed to high concentrations of sediment elutriate showed higher mortality rates and, heart differentiation delays.

Abstract

The Tijuana River Estuary (TRE) is the largest intact wetland in Southern California, and is home to a variety of native and migratory species. It has been historically polluted, and this study aims to evaluate the toxicity potential of estuarine pollutants. Estuarine sediment was used to prepare elutriate with 15 ppt saltwater. Thirteen groups of medaka fish (*Oryzias latipes*) embryos were collected from six adult females and split into five exposure groups (0%, 10%, 50%, 75%, and 100% elutriate concentration). The embryo development and heart physiology were recorded at 3 and 7 days post-fertilization (dpf). Embryos exposed to higher concentrations of elutriate had higher mortality and delayed heart differentiation compared, suggesting possible developmental toxicity of sediment-bound pollutants.

Introduction

Background

- Tijuana River Estuary (TRE) connects the Tijuana River and the Pacific Ocean[1], where tidal cycles affect salinity, nutrient content, and toxicant accumulation in the brackish environment[2].
- Toxicants such as banned pesticide DDT have been found at the TRE, sparking concerns for the well-being of the public and the ecosystem[3] [4].
- Medaka fish serve as suitable model organisms for simulating TRE conditions.
  - Transparent eggs with well-characterized developmental stages
  - Tolerance to salinity fluctuations[5]

Significance

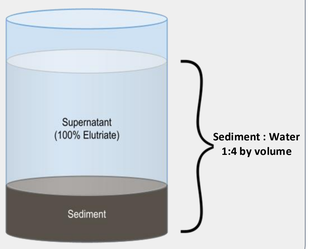
- Pollution affects water quality and subsequently native organisms and local communities in the Tijuana River Watershed (Figure 1) in both the United States and Mexico [2].

Figure 1. Tijuana River Watershed, including all tributaries of the Tijuana River. William Bay, Tijuana River National Estuarine Research Reserve.

Materials and Methods

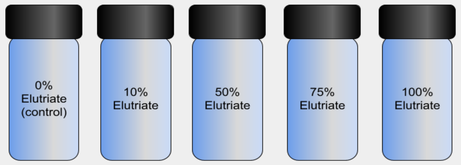
Elutriate Preparation

- Sediment sample collected from TRE at 32.547251, -117.064224
- Mixed sediment and 15 ppt salt water at 1:4 ratio
- Let mixture settle; siphon and centrifuge supernatant to collect sediment elutriate



Embryo Treatment and Observation

- Prepare 5 treatment solutions with sediment elutriate and 15 ppt salt water:



- Set up exposure: 4 ml of treatment solution and one embryo per 8 ml glass vial
- Incubate embryos at 26 ± 1°C with 14 h light / 10 h dark cycle
- Score embryo development and measure heart rate at 3 and 7 days post fertilization (dpf)

Data Analysis

- Used R (v 4.4.3) for data processing, analysis, and visualization
- Heart differentiation status was scored as 0 = tubular, 1 = partially differentiated, and 3 = fully differentiated heart
- Performed One-way ANOVA to compare heart differentiation scores across experimental conditions

Results

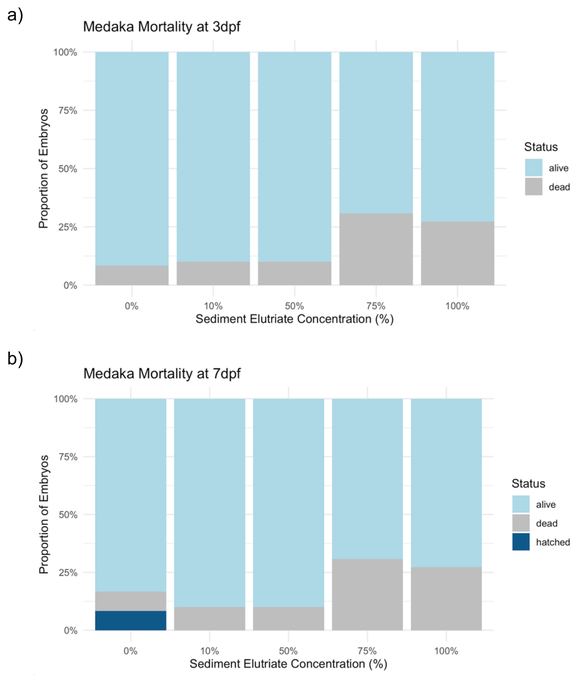


Figure 2. Medaka embryo survival status by experimental condition at (a) 3 days post-fertilization and (b) 7 days post-fertilization.

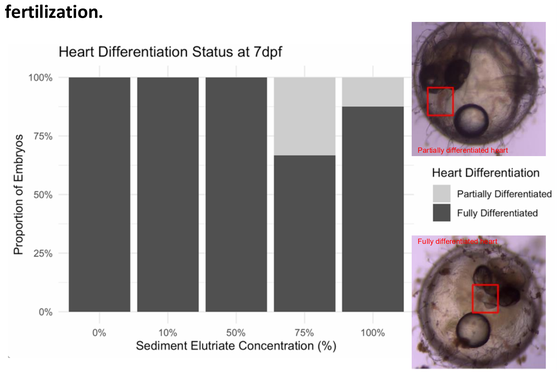


Figure 3. Medaka heart differentiation status by experimental condition at 7dpf. A fully differentiated heart had two obvious chamber. By 7dpf, medaka embryos are expected to have a fully developed heart[6]. The heart differentiation scores were significantly different among the groups (One-way ANOVA, $F = 2.676$, $p < 0.05$), but not significantly different between any two groups.

Discussion

Sediment elutriate exposure effects

- Embryos exposed to higher concentrations of sediment elutriate (75% and 100%) had higher mortality rates compared to others (Figure 2), and the ones who survived the exposure had slower heart differentiation (Figure 3).
- There is minimal change in mortality rates from 3 dpf to 7 dpf.
  - The potential embryotoxicity likely has a larger impact during early development (Stages 1-24).
- Heart differentiation is a key indicator of embryo development.
  - Typically, heart differentiation in medaka occurs around stage 29[8].
  - At 7dpf, all the embryos were between stage 36 and 38. The partially differentiated hearts at 75% and 100% concentration highlights a critical developmental delay.

Sources of variations in data

- Some of the variations in mortality rate may be influenced by genetic variations or technical errors
  - All the embryos in one clutch died (n = 3 for 75% exposure and n = 1 for all other conditions, accounting for 12.5% of all embryos tested), regardless of sediment elutriate concentration.
- At 7 dpf, one embryo in the control group hatched (Figure 2b).
  - Medaka embryos typically hatch around 10 dpf, but have a large natural range of variation, with some taking more than two weeks to hatch.
  - Higher temperatures tend to increase developmental rate[7].
  - This early hatching individual may have been affected by uneven distribution of heat in the incubator, or handling during microscopic observations.

Study Limitations and Future Directions

- Embryos were collected from six medaka mothers, with each clutch spread across exposure conditions.
  - The relatively low number of eggs per condition in each trial limited statistical power
- Specific toxicants that have been detected in the estuary could be individual tested.
  - Previous studies have shown mercury[7] and tributyltin [7] interfere with medaka embryo development.
  - Testing specific chemicals individually can help futher determine the mechanism of observed exposure effects.

References

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