Delayed medaka embryos development and heart differentiation following exposure to Tijuana River Estuary sediment elutriate

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Summary

- The Tijuana River Estuary has been polluted by waste, sewage and chemical runoff, threatening neighboring communities and local ecosystem.
- This study explores the exposure effects of pollution from the estuary on the development of medaka fish embryos.
- Medaka embryos were exposed to different concentrations of estuarine sediment elutriate and observed at 3 and 7 days post fertilization (dpf).
- Embryos exposed to higher toxicant levels generally had slower development and heart differentiation, and lower heart rates.
- However, the exposure effects do not follow a clear dose-response relationship, and are less pronounced at 7 dpf compared to 3 dpf.

Abstract

Tijuana River Estuary is the largest coastal wetland in Southern California. Historically, pollutions including transboundary sewage spills have contaminated the estuarine environment. We assessed the impact of toxicants from the Tijuana River Estuary by exposing developing medaka embryos to estuarine sediment elutriate. The sediment was collected from an inland site where pollutant concentrations were high due to limited tidal flushing and accumulated pollutants over time. Embryos were examined for developmental and heart physiology abnormalities at 3 and 7 days post fertilization following exposures to varying concentrations of elutriate (undiluted, 75%, 50%, and 10%). Our results showed exposure to higher concentrations of the elutriates correlated with delayed heart differentiation and lowered heart rate. These findings highlight the urgent need for stronger pollution control measures and ecosystem monitoring efforts to protect the environment and ecosystem in the Tijuana River Estuary.

Introduction

Figure 1. Photo of TRE taken during the field trip.

Tijuana River Estuary (TRE)

- Historically polluted by
  - Industrial waste (PCBs/heavy metal)
  - Untreated sewage
  - Urban runoff and pesticides (1)

Impact on local lives

- Threatens the local ecosystem and public health.
- Popular beaches in the region are impacted from the wastewater, resulting in human illness (2) (3).
- Reports from surrounding communities have recently indicated a worsening of conditions, with a strong, foul odor, raising concerns about the severity of contamination.
- Studies have detected pathogens such as Hepatitis A virus and bacteria from Tijuana sewage in coastal waters (4).

Sources of contamination

- Pollutants originated from many locations (5).
- As the Tijuana River river flows from inland areas toward the Pacific Ocean, it accumulates toxicants (6).
- The river ultimately empties into the Tijuana River Estuary (7)

Toxicity testing with medaka (Japanese rice fish)

- Rapid embryo development: hatch in 1-2 weeks post fertilization
- Genetic similarly to other vertebrates
- Tolerates the salinity fluctuations estuary (8)

Hypothesis

Medaka embryos exposed to more concentrated elutriate derived from Tijuana River Estuary sediment will have slower development because toxicants in the elutriate will impair biological processes such as heart development.

Materials and Methods

Embryo collection:
Embryos were collected from 6 different mothers and bleached

Sediment collection:
Sediment collected from the Tijuana River Estuary at 32°32'47.49", 117°03'57.7476"W

Embryo exposure:
Each individual embryo was placed in a glass vial with 4 ml exposure solution: 10%, 50%, 75%, 100% elutriate, or control (15 ppt brackish water)

Sediment elutriate preparation:
Elutriate prepared by mixing sediment with brackish water (salinity = 15 ppt), adapted from the EPA Standard Elutriate Test protocol
The elutriate was then used for preparing embryo exposure solutions

Observations:
Embryo development was assessed at 3 and 7 dpf for
- Developmental stage
- Heart morphology
- Heart rate

Embryos frozen at -80°C for RNA extractions

Data analysis:
- Heart morphology was scored as 0 = tubular, 1 = partially differentiated, and 3 = fully differentiated heart
- Data processing, analysis, and visualization was performed in R (v 4.4.3) via tidyverse (v 1.1.4) and ggplot2 (v 3.5.1)
- One-way ANOVA was performed to compare developmental stage, heart morphology score, and heart rate differences across exposure groups

Results

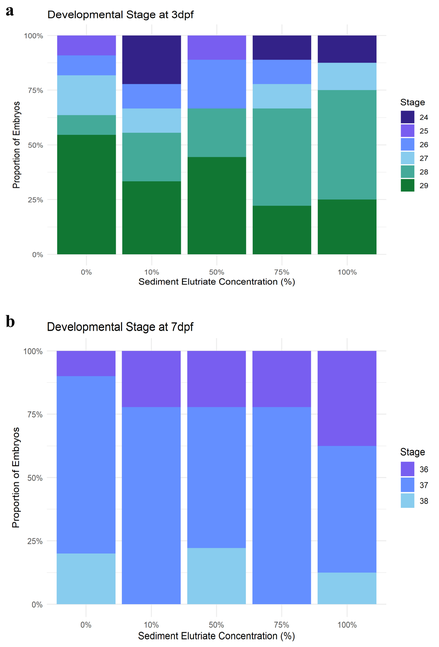

Figure 2. Developmental stages of embryos per exposure condition at (a) 3 and (b) 7 days post fertilization. Dead embryos were excluded. n = 8~11 per condition. There was no statistically significant developmental stage difference among the exposure groups (One-way ANOVA, $p > 0.05$).

Results

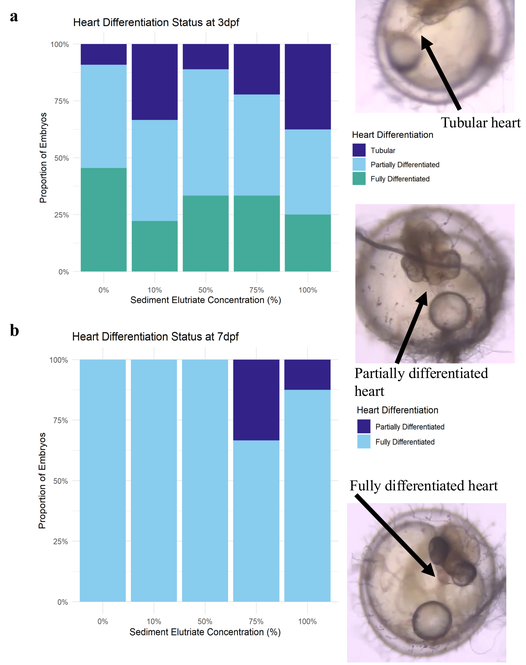

Figure 3. Heart morphology per exposure condition at (a) 3 and (b) 7 days post fertilization. The 7 dpf heart differentiation scores were significantly different among the groups (One-way ANOVA, $F = 2.676$, $p < 0.05$), but not significantly different between any two groups.

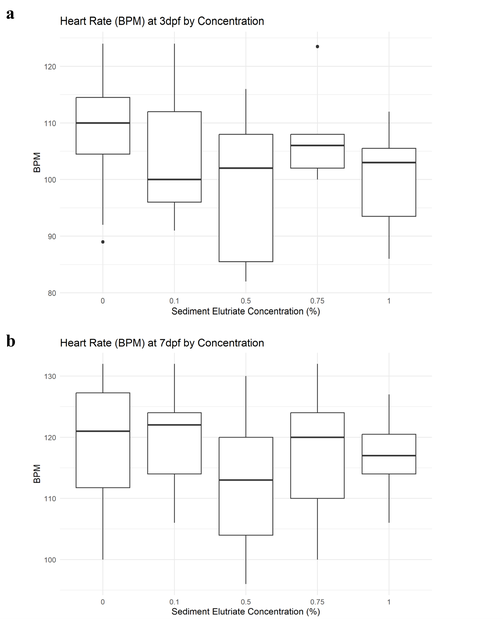

Figure 4. Embryo heart rate per exposure condition at (a) 3 and (b) 7 days post fertilization. There was no statistically significant heart rate difference among the exposure groups (One-way ANOVA, $p > 0.05$).

Conclusion/Discussion

Exposure to concentrated sediment elutriate slightly disrupted medaka embryo development, including delayed heart differentiation and lower heart rate.

- Considering developmental stage and heart differentiation status, there was a minor difference between the control and exposed groups, especially at 3 days post fertilization (dpf).
  - The control group had more embryos at later developmental stages (Figure 2a) and fewer embryos with underdeveloped (tubular) heart (Figure 3a) at 3 dpf.
  - We expected higher exposure levels to correlate with slower heart rate, medaka embryos exposed to the toxic tributyltin had slow heart beats (9).
  - Nevertheless, the slower heart rates among exposed group embryos at 3 dpf (Figure 4a) are likely due to their less developed hearts instead of direct toxicological effects.
- Unexpectedly, embryos exposed to 50% elutriate had the most similar heart differentiation score distribution as the control group (Figure 3a), and there was only one embryo in each group that had a tubular heart.
  - The was also not a clear dose-response relationship. While at 7 dpf, only embryos in the 75% and 100% exposure groups still have partially differentiated heart (Figure 3b), their overall development is similarly slow as embryos exposed to 10% elutriate (Figure 2b).
  - Notably, embryos in the 10% and 100% exposure groups had the slowest heart differentiation and heart rate at 3 dpf (Figure 3a, 4a).
- Although the differences were not statistically significant, the exposed groups together display consistently slower development at both time points, and slower heart rate at 3 dpf.
  - These effects are less pronounced at 7 dpf.
- The exposed groups also have higher mortality rates than the control.
- Our results demonstrate the potential effect of sediment-bound pollutants on fish embryo development and highlight the ecological risks posed by contaminated water in the Tijuana River Estuary.

Study Limitation and Future Directions

- Only about 10 embryos were examined for each experimental condition.
  - It takes medaka embryos around 10 days to hatch after fertilization under our incubation conditions, but their natural growth rate is highly varied among individuals.
    - One of the embryos in the control group hatched at 7 dpf, and it can take more than two weeks for slow-growing embryos to hatch.
  - More replicates can mitigate possible human errors during experiments and increase statistical power.
- In our data, the differences between control and exposed embryos are more consistent at 3 dpf.
  - Exposure effects are likely to vary depending on exposure duration and developmental stage.
  - Future experiments can explor long-term exposure effects beyond the embryo stages, such as hatching rates and larvae survival rates.
- Comprehensive assessment of other critical organs (such as brain and gill) and behavior/locomotion can better determine the systemic effect of exposures.
- Other locations with different pollutant compositions should be tested to evaluate pollution impact on local aquatic species across the Tijuana River Estuary.

References