Polluted Tijuana River Estuary Sediment Elutriate Exposure Results in Delayed Cardiac Morphogenesis in *Oryzias latipes*

Zonca, I.[1], Jadidi, S.[1], Ye, G.[2], Muhammad, U.[1], Lu, J.[3], Vasquez, M.[3], Feng, Z.[3] Bozinovic, G.[3]

University City High School[1], Del Norte High School[2], Boz Life Science Research and Teaching Institute[3]

Summary

- The transboundary Tijuana River has been historically polluted by pesticides, PAHs, and phthalates, which have been associated with the cardiothoracic health issues.
- Research question: how do varying concentrations of pollutants from the Tijuana River Estuary affect heart differentiation in the medaka fish (*Oryzias latipes*)?
- Sediment elutriates were prepared with estuarine samples and used for fish embryo exposure.
- Heart differentiation was delayed with higher sediment elutriate concentration.

Introduction

Hypothesis: higher sediment elutriate concentrations cause increased frequency and severity of cardiac malformations in medaka embryos.

- The Tijuana River runs from Tijuana, Mexico to San Diego, U.S. This major freshwater source for the Tijuana River Estuary (TRE) has been historically polluted by polluted by urban runoff, industrial and agricultural waste, sewage, and trash
- Major contaminants include fecal bacteria from wastewater, polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorinated biphenyls (PCBs), phthalates, and heavy metals (Musegaas 2024; McLamb et al. 2023).
- Sediment samples from sites upstream along the Tijuana River close to the border have especially high PAHs levels, especially during dry periods (Musegaas 2024; McLamb et al. 2023).
- PAHs, heavy metals, and endocrine disruptors are known to disrupt fish heart development (e.g., contractility, calcium signaling, looping) (Musegaas 2024).
- The Japanese rice fish, medaka, are good model organisms for assessing costal estuarine environment because they are native to brackish water.
- Medaka embryos are good developmental toxicity models because they are translucent, have well characterized developmental stages, and develop quickly (Padilla et al. 2009).
- Pollutants in TRE sediments may impair medaka cardiogenesis.

Methodology

1. Sediment Sampling

- Sediment sample from a heavily polluted in-land site close to the border was collected at the end of a dry season.

Site sampling map

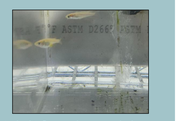
2. Prepare Elutriate

- Frozen sediment was thawed to room temperature, combined with 15 ppt saltwater in equal amounts, mixed for 30 minutes, and settled for an hour.
- Isolated supernatant as the final elutriate.

Thawing sediment sample

3. Collect Medaka Embryos

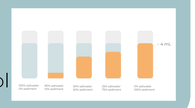
- Collected within an hour after the onset of the light cycle in the morning.
- Used a plastic pipette to collect embryos from the mothers.



Adult female medaka cultivated at Boz Institute

4. Setup Exposure

- Split clutches of embryos evenly across different treatments: 10%, 50%, 75% elutriate diluted with 15 ppt saltwater, along with a control condition with just the saltwater.



5. Monitor Embryo Development

- Scored embryos at 3 and 7 days post-exposure
- Significant differences in developmental stages, heart differentiation, and heart rate were tested with Kruskal-Wallis tests followed by Dunn's tests with Bonferroni corrections

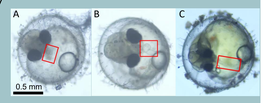


Figure 1. A) Fully differentiated, B) partially differentiated and C) tubular hearts.

Results

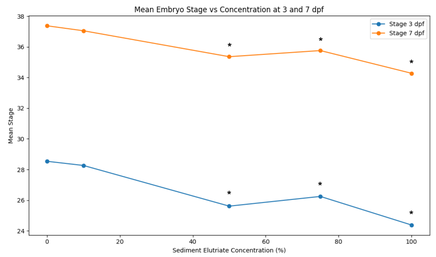


Figure 2. At both 3 dpf and 7 dpf, higher elutriate exposure resulted in more delayed development. * indicates significantly differences in developmental stage relative to the control group ($p < 0.001$).

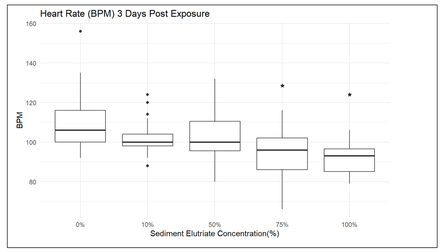


Figure 3. At 3 dpf, heart rate is generally lower with higher sediment elutriate concentration. * indicates significantly lower heart rate relative to the control group ($p < 0.001$).

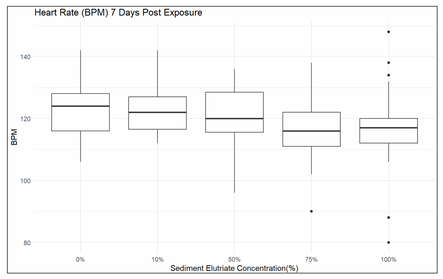


Figure 4. By 7 dpf, embryos in higher elutriate concentrations tend to have generally lower, though not statistically significantly different heart rates compared to the controls.

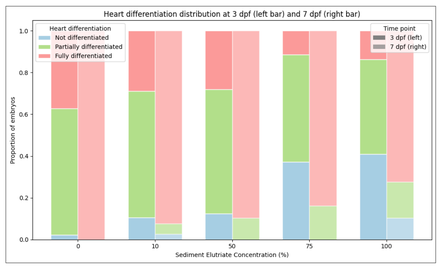


Figure 5. The proportion of non-differentiated hearts increased and the proportion of fully differentiated hearts decreased with higher elutriate concentrations at 3 dpf.

Discussion & Conclusion

Our hypothesis is supported by the dose-response effects observed:

- As sediment elutriate concentration increase from 0% to 100%, a significant dose-dependent developmental delay effect was observed at both 3 days post-fertilization (dpf) and 7 dpf (Figure 2).
- Embryos in 75% and 100% exposure groups have lower heart differentiation scores at 3 dpf. By 7 dpf, the differences in heart development between groups are less pronounced and the differentiation scores are no longer statistically significantly different (Figure 5).
- Slower heart rates as elutriate concentration increases is a consistent trend across both timepoints (Figure 3-4), but not as pronounced at 7 dpf.
- Slower heart rates may also reflect delayed cardiac development (Fig. 4) as the heart in earlier developmental stages beats slower (Iwamatsu, 2004)
- The cardiac physiology effects are most evident at 3 dpf, during earlier stages of heart development, implying that cardiac development is disrupted early by pollutants in the sediment elutriate.
- Our results highlight the environmental health risks posed by sediment-bound pollutants at the TRE border site.

Study Limitations

- Observation was only at two timepoints instead of continuous monitoring
- Limited resolution with microscopy due to small particles in elutriate.
- Heart differentiation scoring is not standardized

Future Directions

- Evaluate the health of fish native to the estuary
- Analyze successful hatching rate
- Examine organ-specific exposure impacts on lungs, gills, etc.
- Study long-term effects on swimming, feeding, and survival.
- Assess sensitivity to additional stressors (low oxygen, temperature, salinity).
- Identify pollutant mixtures causing the worst effects.
- Use findings to guide cleanup and conservation efforts.

Bibliography