Medaka Embryos Eye Morphology Affected by Exposure to Polluted Tijuana River Estuary Sediment

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ABSTRACT

The Tijuana River Estuary (TRE) is a critical part of the San Diego-Tijuana ecosystem and home to many organisms. TRE has been historically polluted, raising ecological and environmental health concerns. Sediment elutriates were prepared and used to expose medaka fish embryos. The embryos' eye length to body length ratio were measured at two timepoints, 3- and 7-day post fertilization. The medaka eye to body length ratio generally decreased as elutriate concentration increased. The effect is more pronounced at the earlier developmental stage. Our results highlight the sensitivity of fish embryos to pollutants during early developmental stages.

INTRODUCTION

Hypothesis:

- Exposure to polluted sediment elutriate will cause morphological and developmental abnormalities in medaka fish embryos compared to control embryos, and bacterial composition in sediments will reflect the anthropogenic pollution in the Tijuana River Estuary (TRE).

Background:

- The Tijuana River watershed is the drainage basin for the Tijuana River. Groundwater and runoff allow pollution from farms, factories, and urban areas to collect and accumulate at the outlet of the watershed, the TRE.
- TRE is a wetland and riparian ecosystem in Southern California

Medaka Fish:

- The medaka fish's well-established developmental stage markers make them a great model organism for observing developmental abnormalities. Its high salinity tolerance allows it to adapt in variable and brackish waters such as that of the TRE.
- The average diameter of a fully grown medaka's eyeball is 0.4 to 0.5 mm.

Bacteria:

- Bacteria have preferred growth conditions, and the influx of pollutants can change the bacterial composition in an environment.
- In industrial waste and oil spills, hydrocarbon-degrading bacteria like Myxococcota are commonly found; they specialize in breaking down straight-chain alkanes.
- In sewage waste and fecal-contaminated environments, Bacteroidota and Firmicutes are prevalent and firmicutes have reported high tolerance of metal-contaminated soils.

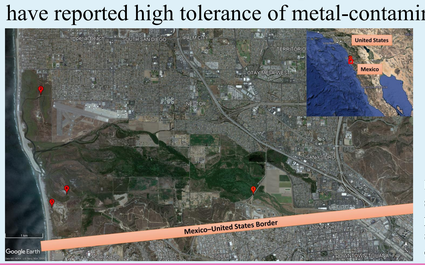

Figure 1. The four sites of sediment collection. Each pin indicates one of the 4 sites where sediment was collected.

METHODOLOGY

Sample Collection and Elutriate Preparation

- Sediments were collected from four sites (Figure 1) at 4 cm deep and stored at -20°C
- Targeted chemical analysis characterized Site 1 sediment as the most polluted, and it is used for the embryo exposure experiments
- Elutriated sediment at 1:1 ratio with 15 ppt salt water

Fish Maintenance and Embryo Exposure

- Medaka were raised in circulating tank system with 14h light/ 10h dark cycles
- Embryos were collected from the abdomen of the mother on the day of the exposure
- Prepare 8mL glass vials filled with 4 mL 0%, 10%, 50%, 75%, or 100% solutions of the elutriate. The 0% solution serves as the negative control. One embryo was placed in each vial.
- Incubated at 26°C for seven days

Eye and Body Length Measurements

- Measured eye width, eye length, and body length in pixels via PixelScale.
- Width and length of the fish eye were averaged for a single value of the eye size measurement
- Ratio of eye length to body length per developmental stage was calculated as the average eye length divided by average body length

Figure 2. Embryo side view measurement photo. The blue and red lines representing eye length and width, respectively

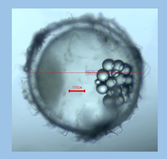

Figure 3. Embryo scale photo The embryo was photographed under 4x magnification with SeBaView.

16S Metagenomics

- DNA extraction from sediment with MOBio PowerSoil DNA Isolation Kit
- Libraries of amplified 16S V4 region sequenced on Illumina NextSeq2000 at Scripps Research Genomics Core

Data Analysis

- Used R (v 4.4.3) for data processing, analysis, and visualization

RESULTS

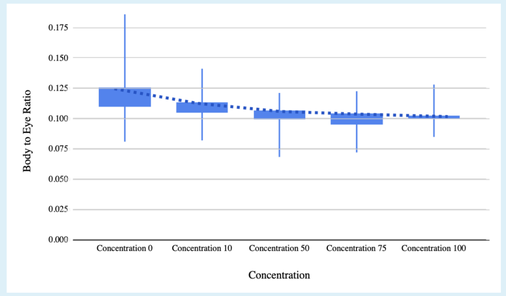

Figure 4. Eye-to-body length ratio decreases as elutriate concentration increases at three days post-fertilization (dpf).

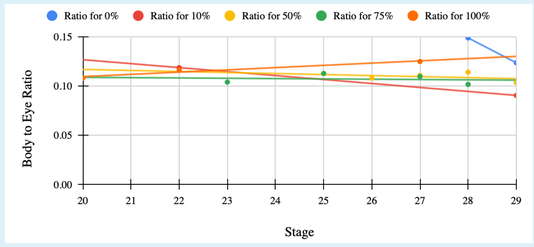

Figure 5. Eye-to-body length ratio across developmental stages at 3 dpf.

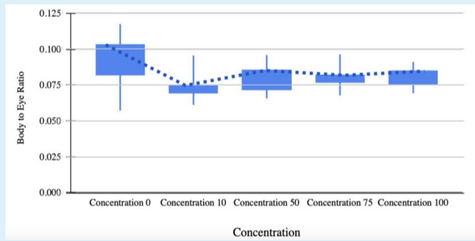

Figure 6. Eye-to-body length ratio decreases as elutriate concentrations increases at 7 dpf.

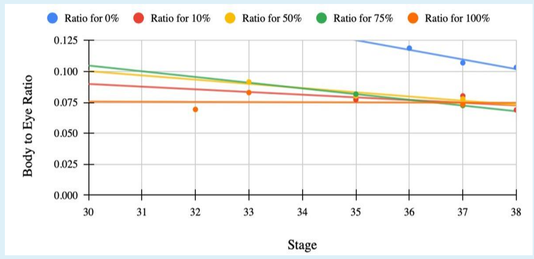

Figure 7. Eye-to-body length ratio tend to decrease in more developed embryos at 7 dpf.

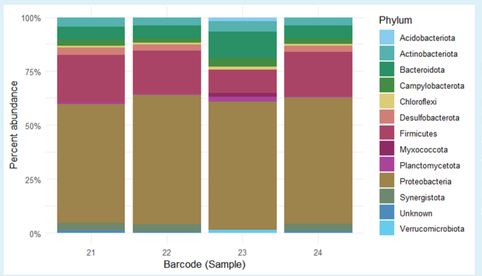

Figure 8. Proteobacteria, Firmicutes, and Bacteroidota are the most abundant bacteria phyla in Site 1 sediment.

DISCUSSIONS & CONCLUSIONS

- Previous chemical analysis showed high PAHs and pesticides levels in Site 1 sediments
- PAHs and crude oil have been known to stunt eye growth due to oxidative stress and induced apoptosis of retinal cells; this is consistent with our findings that body to eye ratio generally decrease more significantly for embryos exposed to higher concentrations of elutriate (Fig. 4 & 6)
- Unlike at 3 dpf (Fig. 4), the eye-to-body ratio at 7 dpf does not have a consistent negative trend across exposure concentrations (Fig. 6), which could be a result of decreased sensitivity to pollutants in later development.
- At 7 dpf, embryos in all treatments have similar trends of lower body-to-eye ratio in later developmental stages (Fig 7), indicating slower growth of eye size in comparison to growth of body size from Stages 30 to 38.
- This pattern is less consistent at 3 dpf, with embryos in the 100% exposure group having the opposite trend (Fig. 5). There are also no embryos in this group that developed beyond Stage 27 at 3 dpf, which may reflect a major delay in eye development in embryos that are also delayed in overall development.
- In fish, thyroid hormone plays a role in eye development, eye size and neurological development. Studies have shown that the inhibition of enzyme thyroid peroxidase (TPO) altered vision-related gene transcripts for fish resulting in disruption of eye size and retinal structures. TPO inhibition also leads to reduced levels of T3 and T4 in fish resulting in reduced growth rate, impaired skeletal development, and reproductive toxicity
- Medaka eyes are homologous to the human eye; both are composed of three nuclear layers with. Photoreceptor rod and cone cells in the outer nuclear layer, Muller glial cells in the inner layer, and ganglion cells in the ganglion cell layer. The changing body to eye ratio for medaka fish could help us predict the effects of the Tijuana River Estuary pollution on the human body upon exposure.
- This most abundant bacteria were the Proteobacteria, which are common in the brackish estuarine environment such as TRE. The second most abundant bacteria were the Firmicutes, which can be commonly found in sewage and wastewater, indicating likely sewage contamination.

LIMITATIONS

- We measured body of the embryo manually and accounted curvature by summing the measurement of small segments, which could have introduced technical error.
- This measurement was taken at least twice and averaged to increase accuracy.

SCAN FOR REFERENCES

SCAN FOR LENGTH MEASUREMENTS