

Assessing the Accumulation of Polycyclic Aromatic Hydrocarbons (PAHs) to Determine the Impact of Urban Pollutants on the Redside Dace (*Clinostomus elongatus*)

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Background & Significance

The Redside Dace (*Clinostomus elongatus*) is an intolerant fish species significantly affected by urbanization in the Greater Toronto Area (GTA), leading to its endangerment in Canada, while maintaining a stable population in Ohio.^{1,2,3,4} Polycyclic aromatic hydrocarbons (PAHs) are an urban pollutant that accumulate in sediment, impacting aquatic organisms and their CYP1A protein expression.^{5,6,7,8} Currently, there is no toxicological or molecular data associated with the Redside Dace that exists, warranting an investigation into the accumulation of PAHs in their habitats and their molecular impact on this species.⁵



Figure 1: Redside Dace (*Clinostomus elongatus*)

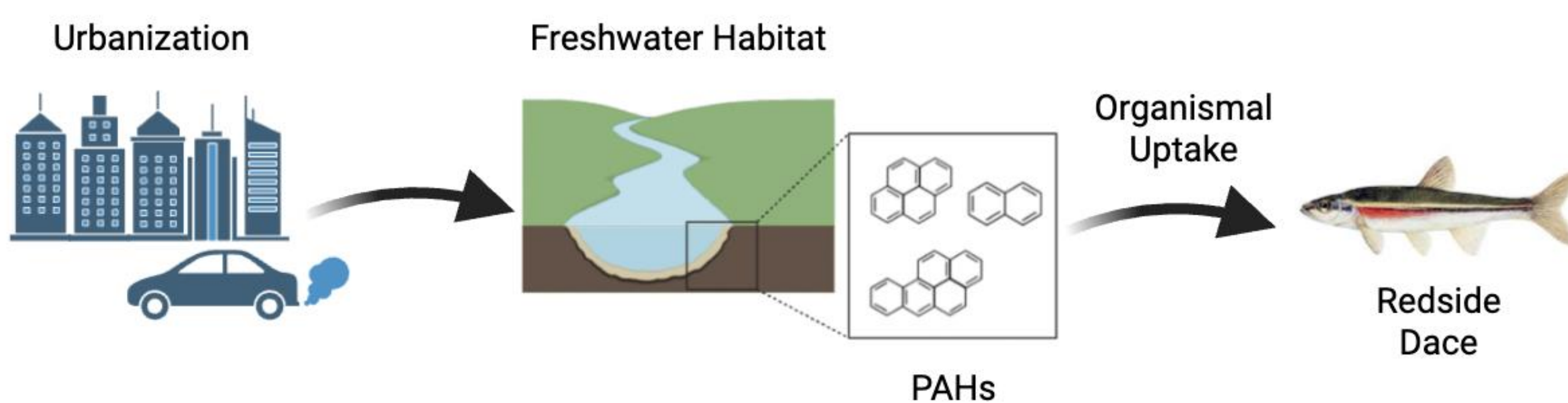


Figure 2: PAHs produced from urbanization accumulate in sediment in freshwater habitats and can be introduced to fish species.

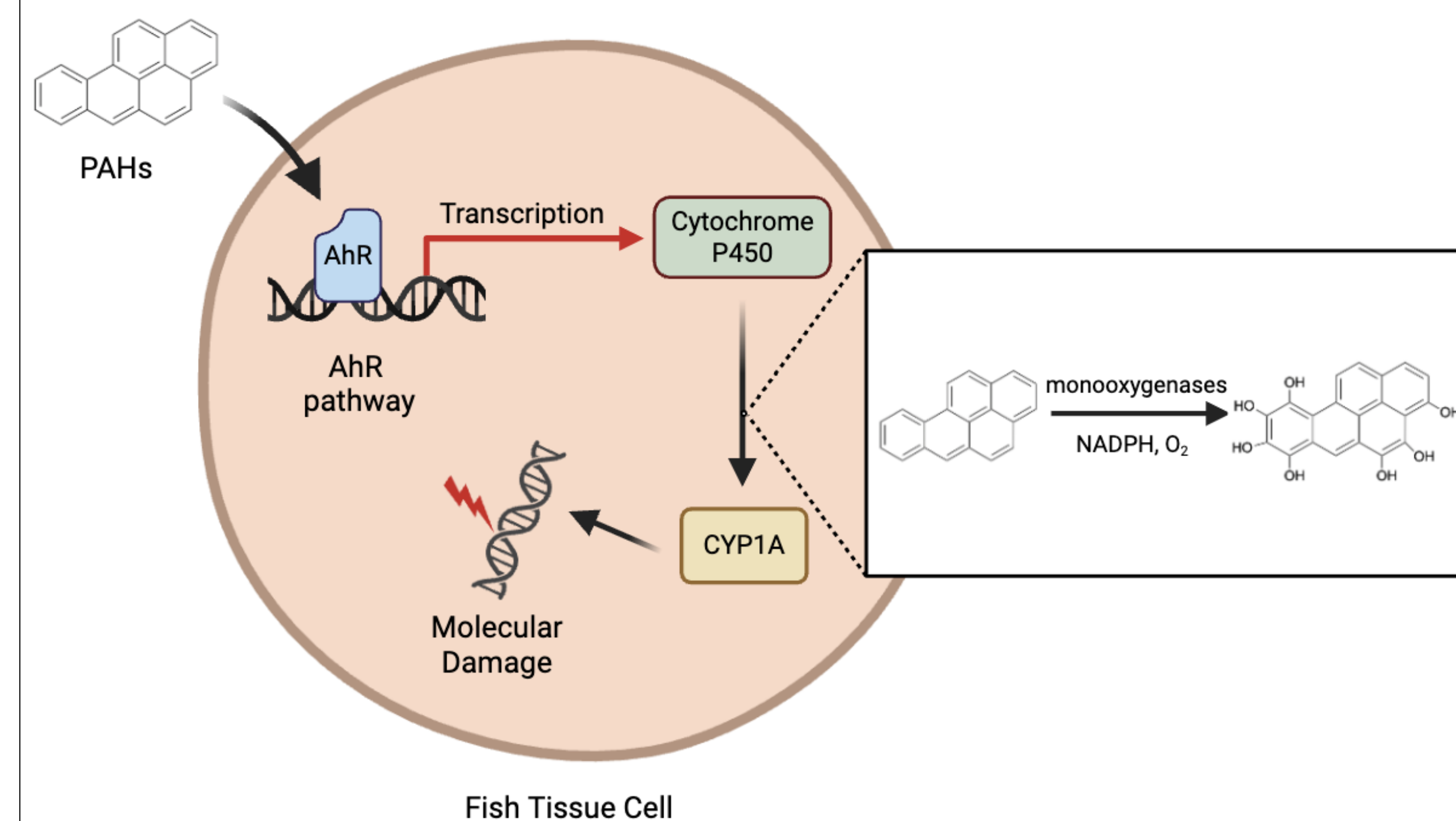


Figure 3: PAHs trigger the aryl hydrocarbon receptor (AhR) pathway, leading to expression of the CYP1A enzyme for metabolic breakdown.

Hypothesis

- Accumulation of PAHs would be higher in sediment collected from aquatic sites in the GTA, where the Redside Dace is endangered.
- Upon exposure to a PAH, benzo[a]pyrene, the Redside Dace will likely reveal CYP1A expression.

Methodology

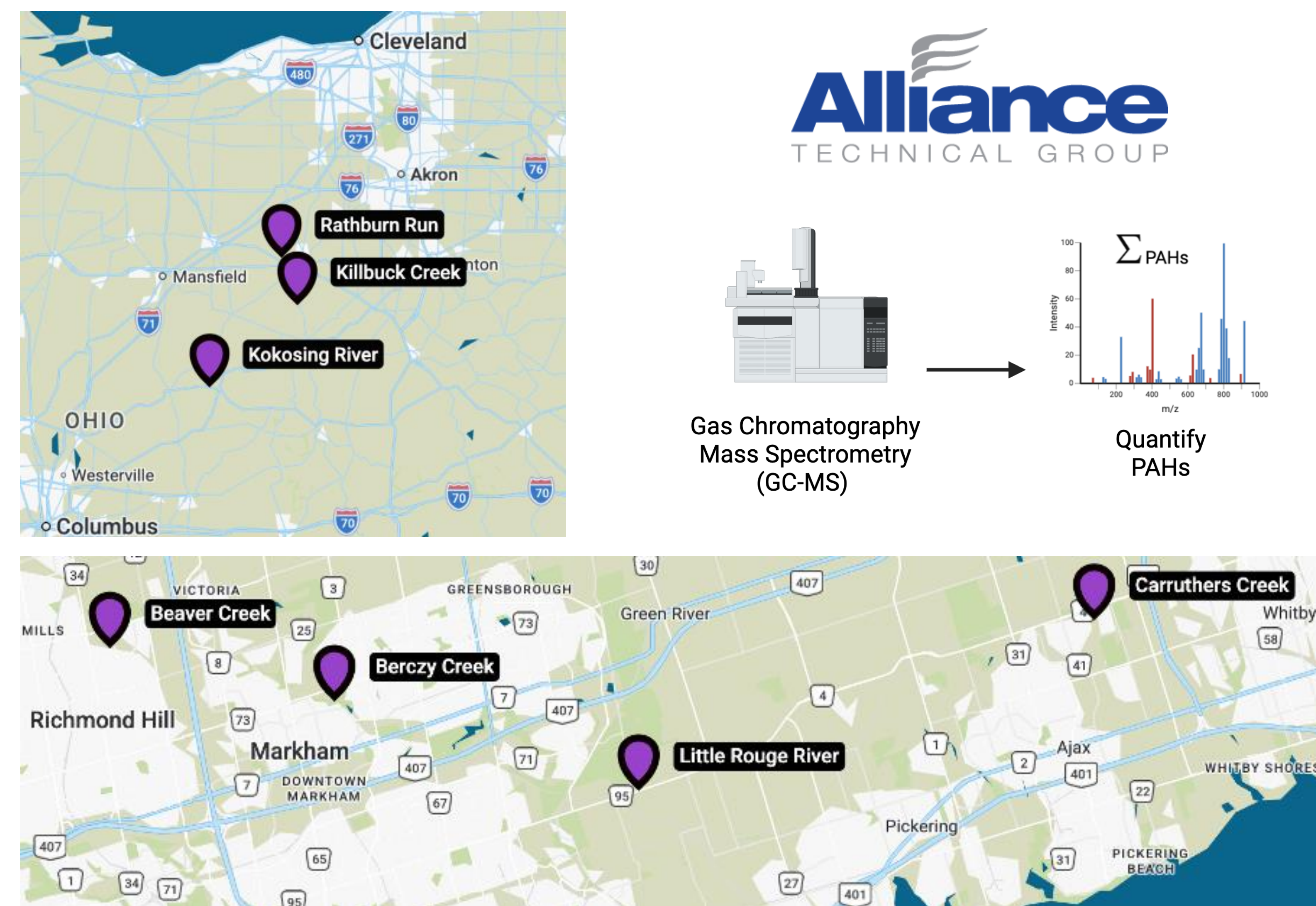


Figure 4: Experimental Methods & Sediment Sampling Sites in Ohio and the GTA

- Sediment samples were collected from three sites in Ohio and four sites in the GTA.
- Alliance Technical Group in Cuyahoga Falls, Ohio conducted GC-MS following EPA Method 8270E.

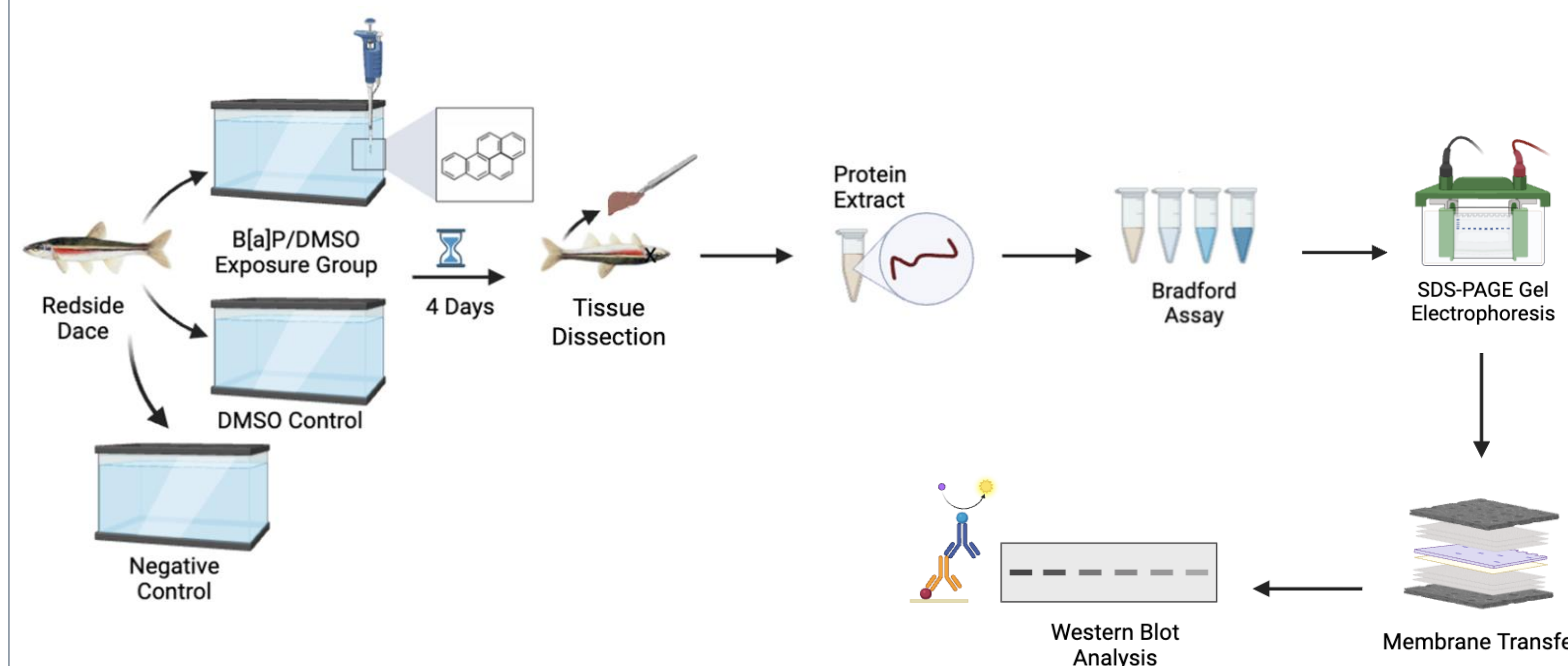


Figure 5: Experimental Methods for the 96-hour B[a]P Exposure Study

- Redside Dace were captured from the Rathburn Run in Wooster Memorial Park in Wooster, Ohio, and acclimated for two weeks.
- Fish were aqueously exposed to 10 µg/Liter of benzo[a]pyrene (B[a]P) in 0.01% in dimethyl sulfoxide (DMSO) for 96 hours.
- Protein was extracted from tissue samples for the detection of CYP1A via Western blot analysis.

Results

- No PAHs or semi-volatile compounds were detected in any sediment samples.
- CYP1A expression was not revealed from chemiluminescent imaging in the B[a]P exposure study but was found in other Redside Dace tissues during troubleshooting.
- Use of a Rhodamine Anti-Actin antibody revealed actin expression in Redside Dace liver and gill tissue samples.

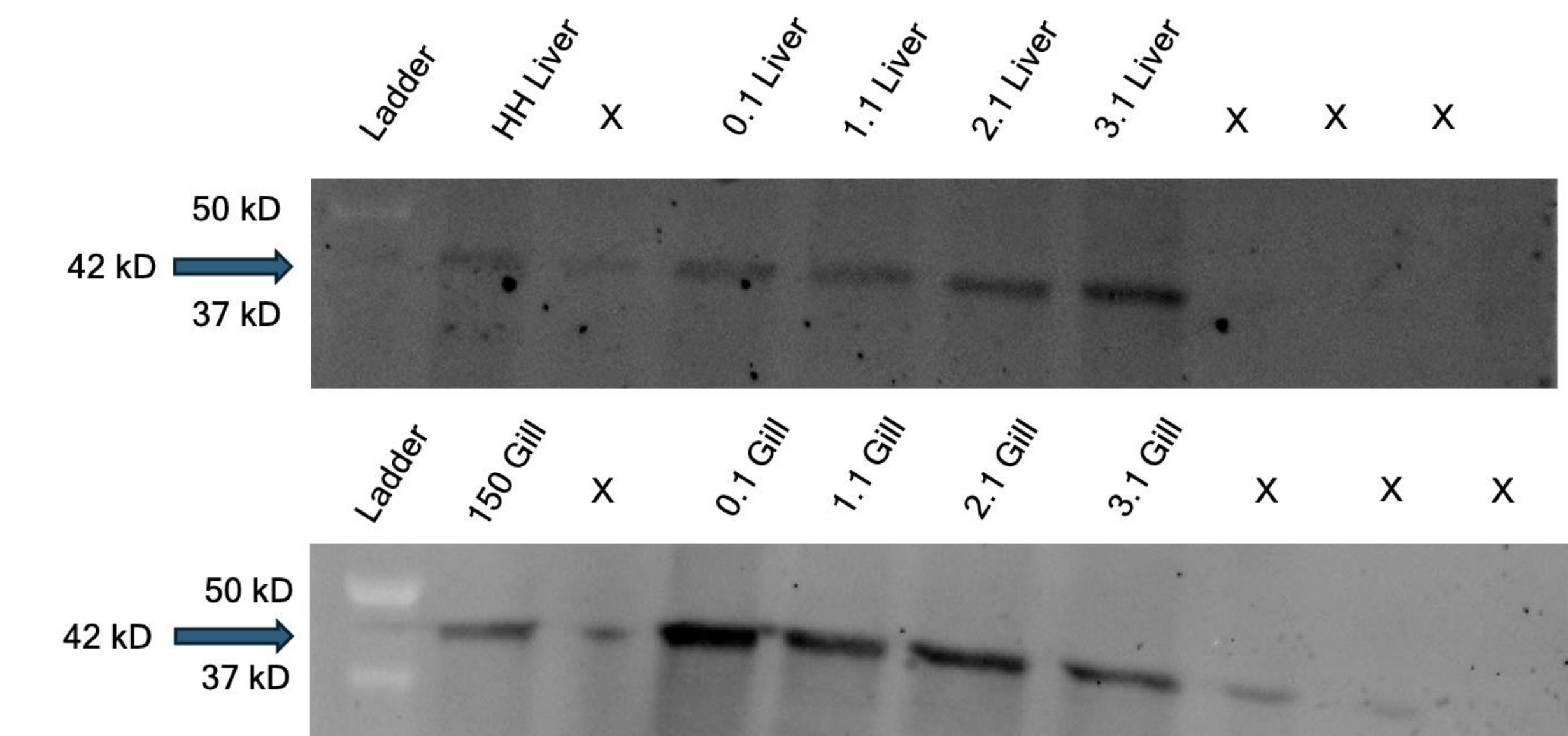


Figure 6: Actin Expression in Redside Dace Liver & Gill Tissue Samples

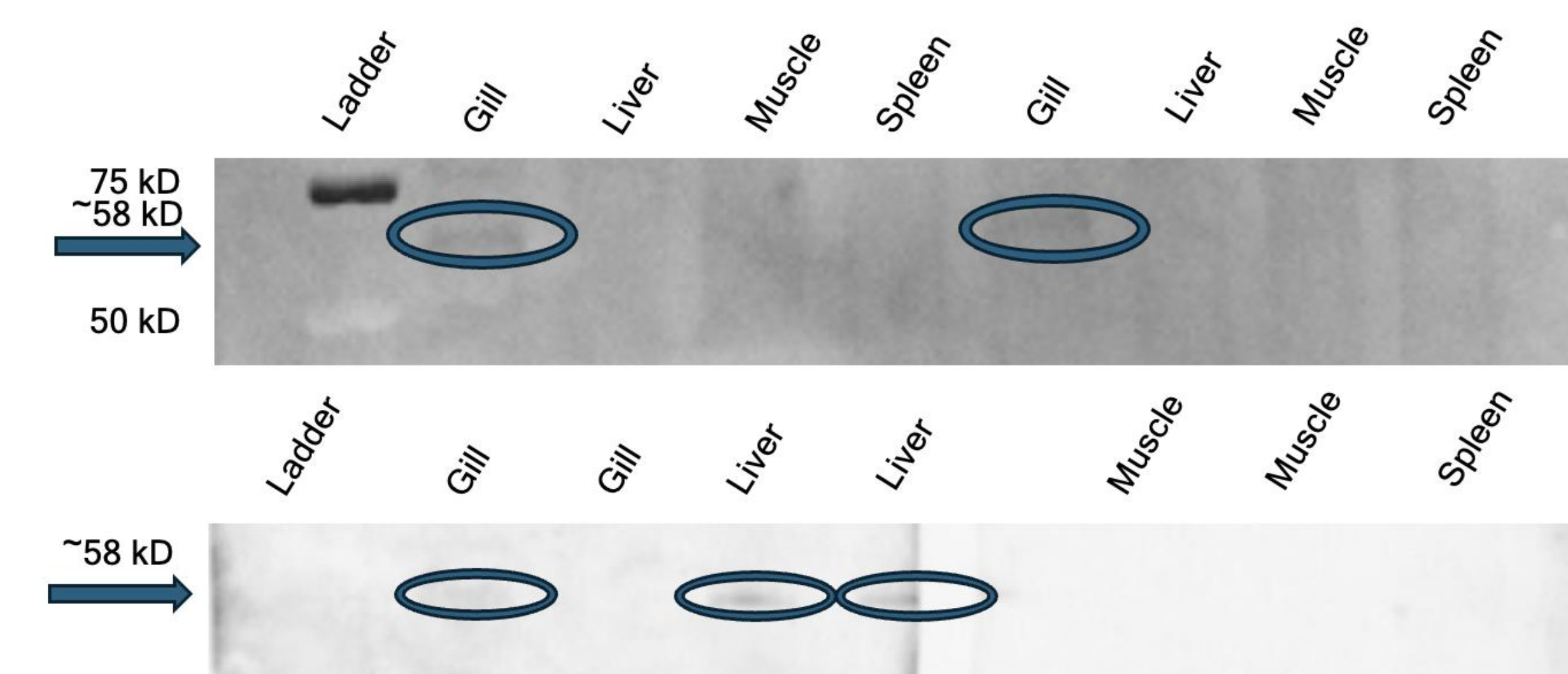


Figure 7: CYP1A detection in Redside Dace Gill & Liver Tissue Samples

Future Directions

- Conjugate a primary CYP1A antibody to improve the efficiency of the Western blot technique.
- Improve the methodology for sediment sampling, such as using a ponar, and investigate other contaminants in Redside Dace habitats.

Acknowledgements

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Literature Cited

