Increased urbanization in Puget Sound leads to elevated enzymatic activity in *Mytilus trossulus*

Ly Vuthy [1], Chris Mantegna [2], Jim Gawel [1], Alison Gardell [1]*
[1] *University Of Washington, Tacoma* , [2] *University of Washington, Seattle*

Introduction

- **Washington Department of Fish and Wildlife (WDFW) Quality Assurance Program Plan (QAPP)–** The WDFW implemented the QAPP in parallel with their regional Stormwater Action Monitoring program (SAM). The QAPP uses a nearshore mussel monitoring system to examine the health of biota in the presence of toxic contaminants in Puget Sound. As SAM continues to make adjustment to the water quality, the Nearshore monitoring program allows them to measure health conditions of organisms overtime.
- **A Model Indicator Species** – Mussels are an ideal indicator species as they have wide distribution across different marine ecosystems, are sessile, and play an important role as ecosystem engineers. As filter feeders, mussels can filter through large volumes of water and accumulate contaminants, making them the candidate organisms to test toxicity responses.
- **Biomarker Expression** - We are complementing WDFW existing work by adding a biomarker component to begin to characterize biochemical response to pollutants in Puget Sound waterways. For this study, we chose to explore the biomarker activity of P450 and SOD. Cytochrome P450 (P450) is a biotransformation enzyme for offloading xenobiotics via detoxification and is expressed when mussels encounter Polycyclic aromatic hydrocarbons (PAHs) and Polychlorinated biphenyls (PCBs). Superoxide Dismutase (SOD) is an enzyme that measures organismal response to oxidative stress. The enzyme catalyzes the conversion of highly reactive radicals to prevent cellular damage.

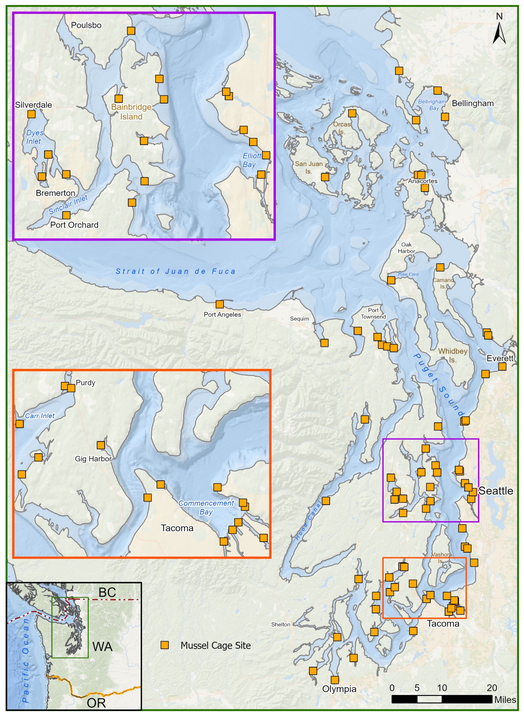

Figure 1. Mussel out planting sites used in 2021/ 2022 WDFW monitoring season. WDFW deploys and retrieves mussels from approximately 150 sites in the Salish Sea region, this map indicates the 74 total sites of the full study. The black bordered map in the bottom left corner of this map shows the out-planting sites at a statewide scale for geographic situation. The map expansions in purple and orange show a clearer view of the Seattle and Tacoma metro area, respectively. Nine of our twelve sites can be viewed these expansions, four in the Seattle area (purple) and five in the Tacoma area (orange).
Map credit: Mariko Langness, WDFW.

Figure 2. Volunteers of the nearshore mussels monitoring program out planting caged mussels at Hylebos site for the 2023/24 season.
Photo Credit: Summer Turnberg

Objective

- Utilize biochemical biomarkers to characterize *Mytilus trossulus* response to urbanization.
- Sites with high urbanization (Commencement Bay, Elliot Bay), minimal urbanization (Hood Canal) and the shellfish aquaculture site were chosen for comparison.
- **Hypothesis: Mussels at sites of higher urbanization show elevated enzyme activity compared to mussels out planted at sites with lower urbanization.**

Methods

- The mussels used in this study were collected from the WDFW's Washington State Mussel Watch Program 2021-2022 survey. Cultured, pre-productive native bay mussels (Mytilus trossulus) sourced from a commercial aquaculture facility (Penn Cove Shellfish, Inc.) on Whidbey Island, WA were transplanted to the monitoring locations. Field and laboratory methods are completely described in the associated SAM program's QAPP (Langness et al., 2022).

Methods Continued

- For both biomarkers, we utilized the sample preparation protocols established and optimized by Counihan et al. (2019).
- The raw enzyme activity was calculated using the standard curve from each plate, normalized to the sample amount used in each assay, and then normalized per milligram of protein.
- Statistical analysis - Biomarker statistical analysis and figure generation were performed in RStudio using R version 4.3.2 and 'tidyR', and 'vegan' packages. Data is not normally distributed and analyzed using the non-parametric Kruskal-Wallis test and Kruskal-Wallis Multiple Comparisons post hoc test from the 'pgirmess' package. Mapping was completed with the 'ggplot2', 'sf', 'tmap', 'viridis', 'rnaturalearth', 'rnaturalearchdata' packages. Scripts available in https://github.com/ChrisMantegna/WDFWmussels

Method Road Map

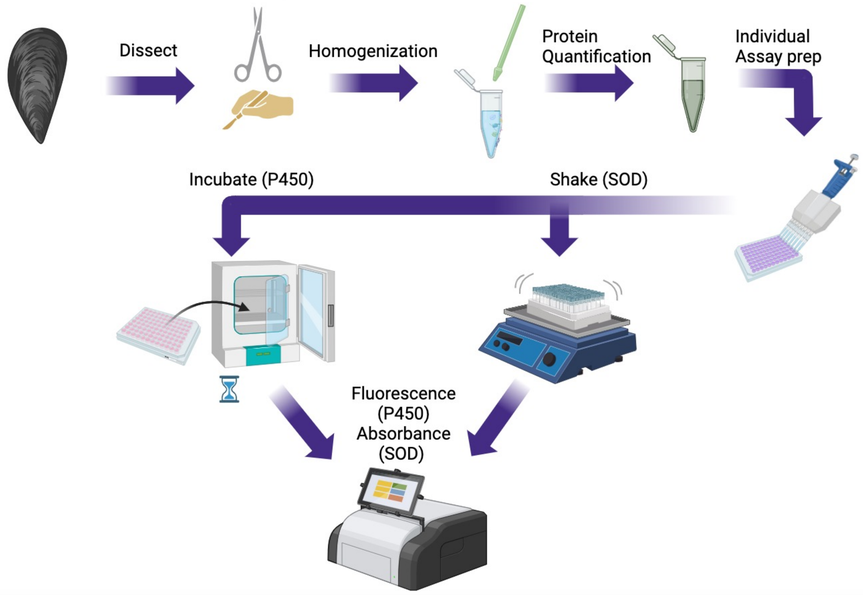

Figure 3. A schematic representation of the methodology used in the study. Each site was individually packaged and had 4-6 mussels. Mussels were thawed and morphometrics measurements were taken. Mussels were dissected into left/right gills, left/right mantle, left/right digestive, abductor, and visceral tissue then flash frozen to await assay preparations. All mussels were subject to the same assays using the digestive tissue. The issue was first homogenized and a bicinchoninic acid (BCA) assay was conducted for protein quantification. The remaining tissue was prepped for P450 using Counihan et al. (2019) protocol and SOD using the Cayman SOD kit. Samples for P450 were incubated for 4 hours at room temperature then placed in the SpectraMax iD3 - plate reader for fluorescence reading at 410 excitation and 530 emissions. Samples for SOD were placed on a shaker at room temperature then placed in the plate reader to analyze the absorbance at 450 nm.
CreatedwithBioRender.com

Results

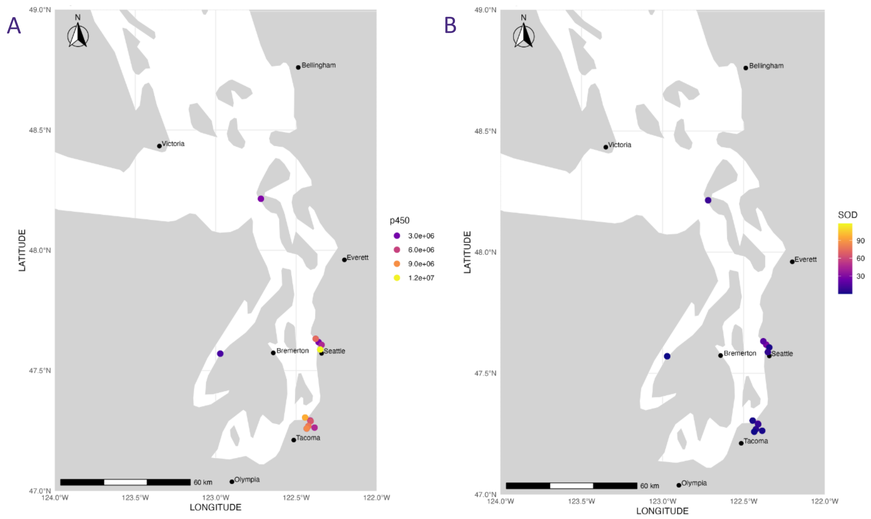

Figure 4A-B. A) Map of P450 activity within mussels in all tested sites. B) Map of SOD within mussels' activity in all sites. Mussels from sites located around Commencement Bay are seen having higher activity of P450. On the contrary, mussels from Elliot bay have higher expressions of SOD.

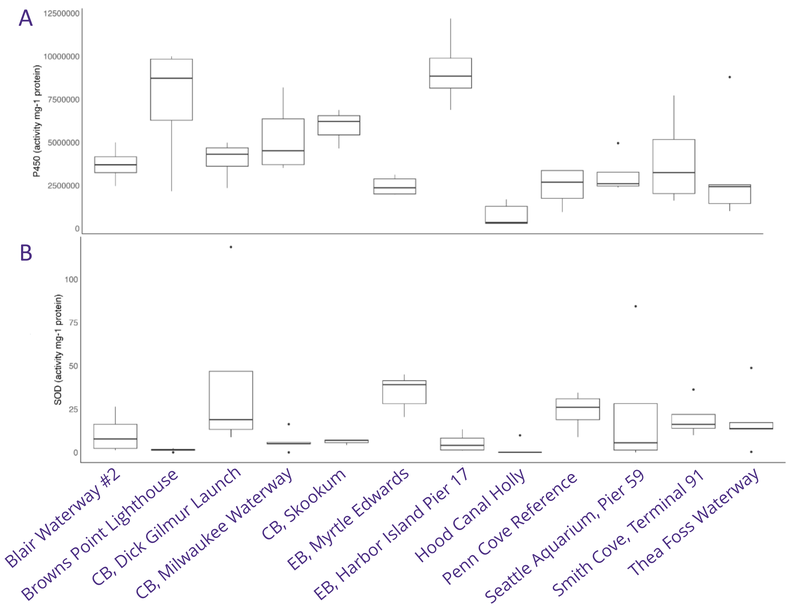

Figure 5A-B. A) Box plot representations of P450 activity at each site. B) Box plot representations of SOD activity at each site. General patterns observed between box plots showcase higher levels of activity mg-1 protein in one enzyme and lower activity in the other. Possible explanation may be due to different contaminants profiles at each site.
CB: Commencement Bay, EB: Elliot Bay

Conclusion

- Mussels found in Elliot Bay, Harbor Island Pier 17 have the highest P450 activity and, which may be related to higher levels of PAHs and/or PCBs in the tissues.
- Mussels found in Elliot Bay, Myrtle Edward have the highest activity of SOD activity, this showcases that the mussels may be under higher stress to combat free radicals.
- Hood Canal mussels demonstrated the lowest activity of both P450 and SOD activity, meaning this site has less pollutants and is less urbanized in comparison to the other sites within the study

Next Steps

- Continued exploration of biomarkers with correlation to existing whole-body burden of contaminants
- Correlation of organismal health and human impact such as potential economic and social impacts - our region relies heavily on the availability of various sources of seafood and other coastal resources for human consumption
- Inform public policy on industrial chemical use, discharge monitoring, and mitigation, and future clean up of determined high contaminated areas.

Acknowledgements

- Land Acknowledgement: "[Our] campus is situated on the traditional lands of the Puyallup Tribe... we want to acknowledge the lands of the Muckleshoot, Nisqually, Suquamish, Snohomish, Sammamish, Duwamish, and all traditional lands on which we live and work. We want to acknowledge the histories of dispossession and forced removal and reflect on this history in ways that honor the memory of those dispossessed and keep us committed to the cause of social justice for indigenous populations." University of Washington – Tacoma.
- I would like to thank Dr. Alison Gardell for welcoming me into her lab and introducing me to her passion for ecotoxicology, while also providing me with guidance throughout the entirety of my capstone project. I would also like to acknowledge Chris Mantegna for her mentorship and support throughout this process.

References

SCAN ME