Approved.



Master of Environmental Studies

#### **Thesis Prospectus 2023-24**

# Name: Maddie Thompson

**ID Number: A00419422** 

Email: thomad29@evergreen.edu

**Student Final Submission (date):** 

Faculty Reader Approval (date):

# **MES Director Approval (date):**

- Working title of your thesis<sup>1</sup>. Title: The effects of synthetic stormwater components on the expression of the environmental stress gene, *cyp1A* in zebrafish (*Danio rerio*).
- 2) In 250 words or less, summarize the key background information needed to understand your research problem and question.

#### Response:

Environmental contaminants from stormwater are known for having cascading effects on the overall health of aquatic ecosystems. However, there are many novel and poorly characterized organic contaminants present in urban stormwater that need to be further studied to better assess the impacts they may have on aquatic organisms (Du et al., 2017). McIntyre et al. (2023) analyzed untreated and treated urban runoff in the early life stages of Pacific salmon and found that survival and morphological development effects were reduced greatly when runoff was properly filtered using bioretention methods. Many toxicity studies have used zebrafish as a model organism to analyze the effects of pollutants on development, physiology, mortality, and stress. One way to visualize environmental toxicity in aquatic organisms is by analyzing stress gene expression. Zhang et al. (2023) tested zebrafish and found that an emerging contaminant, 6-PPDQ (N-(1,3dimethylbutyl)-N'-phenyl-pphenylenediamine quinone), induced both developmental and phenotypical changes. As best management practices (BMP) for stormwater evolve, new emerging contaminants are being discovered that may further alter BMPs. It is important to identify the mechanisms of stormwater toxicity so that better applications of filtration systems and other bioremediation practices can be developed for stormwater management.

3) State your research question(s).

Response:

What effects do the components of synthetic stormwater have on the expression of the

#### cyp1A stress gene in zebrafish?

 Situate your research problem within the relevant literature. What is the theoretical and/or practical framework of your research problem? Response:

One way to visualize environmental toxicity in aquatic organisms is by analyzing gene expression. Transgenic fish have been used to help quantify specific genes associated with fish physiology, growth, resistance, and survival. Researchers have developed protocols to analyze the expression of the cyp1A gene in zebrafish to assess the toxicity of stormwater runoff. Xie et al. (2023) identified cyp1A as the most frequently used biomarker to monitor specific compounds and contaminants in the environment. Although most studies have examined polycyclic aromatic hydrocarbons (PAHs), 6-PPDQ, and other well-known environmental contaminants, few have focused solely on the diverse components of synthetic stormwater. Synthetic stormwater does not produce observed toxicity in aquatic organisms and often results in just overall mortality. Producing/developing synthetic stormwater that mimics roadway runoff chemistry and toxicity to animals is essential to understanding how to mitigate the effects of stormwater. This project will follow the application of analyzing acute stormwater exposures to further address sublethal effects of stormwater components on a model aquatic organisms. Analyzing genes associated with known toxicological pathways in zebrafish such as the cyp1A gene will allow for a better understanding of sublethal effects and their potential toxicity outcome in freshwater ecosystems. Using each of the noted particle and fluid components of the synthetic stormwater (see list for more information) will continue previous research as well as provide new findings for areas that have not been well studied.

5) Explain the significance of this research problem. Why is this research important? What are the potential contributions of your work? How might your work advance scholarship?

#### Response:

Stormwater has increased in both volume and contaminant load due to the increased growth of roadway infrastructure resulting in the leaching of materials and inputs of contaminants into aquatic environments (Kriech and Osborn, 2022). Both the qualitative and quantitative traits of stormwater need further study at both national and regional levels to better inform site-specific, climatic, and local variables that may influence the toxicity of stormwater (Barbosa et al., 2012). The physical effects of stormwater on the phenology of zebrafish, a model freshwater organism, and other fishes have been studied to better inform BMPs. Xie et al. (2023) identify cyp1A (environmental stress gene) as the most frequently used biomarker to monitor specific compounds and contaminants in the environment. Du et al. (2022) found that the expression of the *cyp1A* and *cyp1B1* genes were down-regulated when exposed to polyhalogenated carbazoles (PHCZs) which induced both cardiotoxicity and behavioral changes in zebrafish. Cyp1A is an inducible gene in the liver and many extrahepatic tissues such as the lungs (Sandosh Padamanabhan, 2014), which can be analyzed to assess the level of expression when exposed to environmental contaminants. This project will address gaps in understanding of the direct influences of environmental contaminants on stress genes in an aquatic organism, zebrafish.

Understanding the mechanisms of environmental stress genes and their phenological effects will allow for better applications of filtration systems and other bioremediation practices involved in stormwater management.

6) Summarize your study design<sup>2</sup>. If applicable, identify the key variables in your study. What is their relationship to each other? For example, which variables are you considering as independent (explanatory) and dependent (response)? Response:

This study will use zebrafish as a model aquatic organism to analyze a specific environmental stress gene, *cyp1A*, to quantify levels of expression under sublethal conditions of 7 different components of synthetic stormwater.

The 7 components are brake fluid, used motor oil, transmission fluid, windshield washer fluid, brake particles, exhaust particles, and tire particles. These sources of pollution will be used as individual components of urban stormwater runoff to test developmental toxicity in zebrafish by analyzing a transcriptional gene.

Synthetic stormwater currently does not produce observed toxicity in aquatic organisms without resulting in overall mortality. Producing/developing a synthetic stormwater that mimics roadway runoff chemistry and toxicity to animals was first completed by collaborators in the lab.

Sublethal and lethal toxicity levels were measured through exposures to help develop concentration curves (LC50s). The LC50 curves were then used to determine concentrations for each component to create a synthetic stormwater solution for the experiment.

(MY PORTION):

Independent variable: Concentrations of 7 different components of synthetic stormwater components (Using sublethal concentration values).

Dependent Variable: The levels of expression of the *cyp1A* gene in zebrafish for each synthetic stormwater component.

There are four different protocols for this project: 1) spawning embryos, 2) exposing embryos to stormwater, 3) DNA extractions, and 4) qPCR analysis. See attached protocols for more details regarding the exact protocols. (Note: protocols will be modified and adapted as needed). To summarize: spawning will occur within 1-2 days prior to exposure. After spawning, exposures will occur for each synthetic stormwater component within 6-12 hours post fertilization to ensure exposures occur at optimal developmental stages for embryos. The stormwater exposures will create the required tissue for collection. Tissue collection will be done for mRNA isolation, extraction, and purification. To perform qPCR, I will be using a real-time instrument per the manufacturer's protocol.

Spawning Protocol <u>SpawningSOP</u> Exposure Protocol <u>ZebrafishProjectCaseStudies\Zfish\_Spawn&Exposure\_PCR\_protocol.docx</u> DNA Extraction Protocol <u>DNA Purification Kits, Products & Free Samples | ZYMO RESEARCH</u>) (Link For DNA and qPCR: will need to modify for Zebrafish as I go <u>C:\Users\thomp\Downloads\Zfish\_Spawn&Exposure\_PCR\_protocol.docx</u> PCR Protocol <u>RT qPCR data analysis protocol-sbMay2023 (1).docx</u> these protocols are designed for Coho and the *CYP1A* gene: <u>Salmonid Primer Design Protocol\_june2023.docx</u> <u>Coho BBB\_RT qPCR\_protocol\_(1).docx</u>

USE: <u>Comparative analysis of two new zebrafish models</u>: <u>The cyp1a low-expression</u> <u>line and cyp1a knockout line under PAHs exposure (sciencedirectassets.com)</u>)

7) Describe the data that will be the foundation of your thesis. Will you use existing data, or gather new data (or both)? Describe the process of acquiring or collecting data<sup>3</sup>.

Response:

For my project, the primary data that will be the foundation of my thesis are results from the qPCR. These data will be used to analyze variation in the amount of gene expression of the *cyp1A* gene under various exposures (7 components of synthetic stormwater, plus a control).

Tissue collection will be conducted for mRNA isolation, extraction, and purification. To perform qPCR, I will be using a real-time instrument per the manufacturers' protocol. The lab has used and has a Thermo Fisher Digital PCR and Real-Time device both of which are required for this project.

 Summarize your methods of data analysis. If applicable, discuss any specific techniques, tests, or approaches that you will use to answer your research question. Response:

First, I will be using a t-test on each of the components compared to the control. Then, I will be using an ANOVA to compare the *cyp1A* expression means of each synthetic stormwater component. I will compare the mean values (in this case it will be the expression of the *cyp1A* gene) to determine the difference between each synthetic stormwater component to see how they vary and compared to a control. In order to run an ANOVA I will test for normality (Shapiro-Wilks test) and equality of variance (Levene test), and use Tukey's Tesst to test for differences between pairs of group means. To compare stormwater components, I will use an ANOVA performed in the statistical software R. All statistical tests will be conducted in R program.

9) Address the ethical issues<sup>4</sup> raised by your thesis work. Include issues such as risks to anyone involved in the research, as well as specific people or groups that might benefit from or be harmed by your thesis work, perhaps depending on your results. List any specific reviews you must complete first (e.g., Human Subjects Review or Animal Use Protocol Form).

Response:

The lab I am partnered with required me to review the Animal Use and Protocol Form they have built into their active research. The ethical issue for the scope of my project is built around using the model aquatic lab organism zebrafish to test responses to. In this case, the results from this study are viewed as beneficial to the science community and public. However, when dealing with animals and in this case zebrafish, it is important to consider if there are other ways to address the research question. For this thesis, there are very few alternative in vitro or in silico methods that are available to directly answer this question which makes this approach more acceptable.

10) List specific research permits<sup>5</sup> or permissions you need to obtain before you begin collecting data (e.g. landowner permissions, agency permits).
Response:

NO NEW PERMITS

The only permits I was required to review are the permits to use lab animals for testing. There are no landowner or agency permits used for this specific project.

 Reflect on how your positionality as a researcher could affect your results and how you will account for this in the research process<sup>6</sup>.

Response:

My role in this research is to determine the effects of 7 different stormwater components on the expression of the *cyp1A* gene n zebrafish. Although this research will be carried out by following strict protocols, there are some biases that should be considered. For the selection of fish used in this study we will be using a random sampling method and pooling the fish samples together when exposing them. I would imagine synthetic stormwater would be highly toxic to fishes and other aquatic organisms. So that my original opinions on stormwater do not influence my results, I will follow strict protocols within the research processes. This will reduce my bias perspective so that the results I collect follow the design and not around my opinion of stormwater.

12) Provide at least a rough estimate of the costs associated with conducting your research, if any. Provide details about each budget item so that the breakdown of the final cost is clear.

Response:

I have been provided with the opportunity to collaborate with WSU's Green Stormwater Infrastructure Facility in Puyallup where my research will primarily be funded through their efforts. However, generating any additional funding is promoted such as any costs for consumables I can generate is encouraged (such as funds from applying to the Student Capstone Fund in Winter Quarter). The largest expense for me will be commuting to the facility from my home to execute the research (see attached excel sheet for the travel expenses which be will partially funded by the MES Thesis Fund).

13) Provide a detailed working outline of your thesis. Response:

# 1. Introduction: paragraphs listed below

- 1. Discuss big-picture impacts and threats of stormwater- components of concern
- 2. What is being done to reduce contaminants and improve filtration of toxins (use toxicity study to demonstrate the importance of proper bioremediation systems)
- 3. Link to studies showing phenological effects on fishes (synthetic components)
- 4. Discuss what was done, ex: For this thesis a scholarly literature review was done to analyze what components of stormwater have been heavily studied. To do so 1) stormwater concerns were researched in the literature, 2) the phenological impacts on aquatic organisms, 3) ways to improve study design/application/target analyses (why might qPCR be most useful)

Literature research showed a lack of knowledge regarding the direct impacts of certain components of stormwater contaminants. Thus, this thesis research

addressed the gap by analyzing a specific environmental stress gene, the *cyp1A* gene, in response to various synthetic stormwater contaminants. The current project has 3 goals:

- Use the zebrafish experimental model to assess the effects of known vehicular runoff contaminants in association with stormwater concerns.
- Analyze if the synthetic components induce the cyp1A gene in zebrafish embryos differently.
- Improve urban stormwater filtration by providing baseline information for emerging environmental contaminants.
- 5. Zebrafish Exposures: toxicity endpoints (images of phenological effects and qPCR results)
- 6. What this study allowed for recap study findings once COMPLETE

#### 2. Literature Review: Introduction to Stormwater Concerns and Big Picture of LR

2.1 Stormwater: Environmental Hazards- roadmap

Stormwater Concerns

- 2.2 Stormwater Contaminants- roadmap
- 2.2.1 Stormwater Contaminants: PAHs, Heavy Metals, 6PPD/6PPD-Q, etc.
- 2.3 Aquatic Organisms: Effects of Stormwater Exposure-roadmap
- 2.3.1 Effects of Stormwater Toxin Exposure
- 2.4 Synthetic Stormwater-roadmap

2.4.1 Synthetic Stormwater Research: Particles and Fluids

# 3. Methodology: Zebrafish Toxicity Exposure

3.1 Zebrafish Husbandry

- 3.2 Determining Concentrations
- 3.3 Exposure Procedure
- 3.4 DNA Extractions
- 3.5 qPCR Runs and Analysis
- 3.6 Statistical Analysis

#### 4. Results:

4.1 Toxicity Results for qPCR: Synthetic Stormwater components 1-7

# 5. Discussion:

- Talk about toxicological effects and other ecological implications this work provides to
- Using the molecular biomarker as a means of measuring toxicity will allow us to understand baseline exposures.
- Using these biomarker results for measuring the effectiveness of stormwater mitigation strategies.
- This study is, to our understanding, the first assessment of the embryotoxicity of synthetic stormwater using a variety of types of vehicular environmental contaminants in the zebrafish model.
- 14) Provide a specific work plan and a timeline for each of the major tasks in the work plan. Be as realistic and specific as you can at this point, including the deadlines for Spring quarter.

Response:

The timeline I have created with my collaborators is built around data collection in Winter Quarter and analysis in Spring Quarter.

This means all the data collection which are the exposures, extractions, and running the qPCR must be done within the timeframe of Winter Quarter. To do so, my lab time will begin at the start of Winter Quarter in January where I will be commuting to WSU's facility in Puyallup. To ensure that I have enough samples to measure I should be doing at least one of the synthetic stormwater exposures each week. There are seven components prioritized in this study which each require 3 replicates. This means over the course of seven weeks I would like to do each synthetic stormwater and its replicates. Doing one synthetic component (with exposure replicates) would give the project wiggle room and time to troubleshoot along the way. This would also provide me with extra time at the end to determine if more sample replicates are feasible and/or required.

Date/Week	Goal	Notes
Winter Week 1	Determine Control Replicates Brake Fluid: Start exposures, 3 replicates, run qPCR	Introduction Draft
Winter Week 2	Motor Oil Fluid: Start exposures, 3 replicates, run qPCR	(Maybe run exposures simultaneously to speed up process).
Winter Week 3	Transmission Fluid: Start exposures, 3 replicates, run qPCR	Methods Draft
Winter Week 4	Windshield Washer Fluid: Start exposures, 3 replicates, run qPCR	
Winter Week 5	Brake Particles: Start exposures, 3 replicates, run qPCR	Final Draft of LR
Winter Week 6	Exhaust Particles: Start exposures, 3 replicates, run qPCR	

Timeline Table with Dates:

Winter Week 7	Tire Particles: Start exposures, 3 replicates, run qPCR	Intended Results Storyline
Winter Week 8	Control Replicates and Determine if more replicates are needed for 7 components	
Winter Week 9	Buffer time/Results	Working Draft of Results
Winter Week 10	Built in Time for Hiccups and Bumps in Project	

15) Who (if anyone), beyond your MES thesis reader, will support your thesis (in or outside of Evergreen)? Be specific about who they are and in what capacity they will support your thesis. If you are working with an outside agency or expert, be specific about their expectations for your data analysis or publication of results. Response:

Dr. Jenifer McIntyre (Associate Professor, School of the Environment Washington State University Puyallup Research & Extension Center) will be providing support for the project. The written components will rely solely on my thesis reader's support. The project expectations are that I answer the specified research question with the option of bringing in other morphological effects the lab may also be running. There are no expectations to publish these findings, but I am open to suggestions if the lab wishes to publish these findings.

This will be determined prior to the start date of the project Winter week 1.

16) Provide the 5 most important references you have used to identify the specific questions and context of your topic, help with issues of research design and analysis, and/or provide a basis for interpretation. Annotate these references with notes on how they relate to/will be helpful for your thesis. For any other sources cited in your prospectus in other answers, provide a complete bibliographic citation here as well. Response:

1. Du, B., Lofton, J. M., Peter, K. T., Gipe, A. D., James, C. A., McIntyre, J. K., Scholz, N. L., Baker, J. E., & Kolodziej, E. P. (2017). Development of suspect and non-target screening methods for detection of organic contaminants in highway runoff and fish tissue with high-resolution time-of-flight mass spectrometry. Environmental Science: Processes & Impacts, 19(9), 1185–1196. https://doi.org/10.1039/C7EM00243B

Annotation: Great study design, this paper will be particularly useful in my literature review for stormwater contaminants.

Environmental contaminants from stormwater are known for having cascading effects on ecosystems and the overall health of the ecosystem. However, there are many novel and poorly characterized organic contaminants present in urban stormwater that need to be further studied to better assess the impacts they have on aquatic organisms (Du et al., 2017).

2. Du, Z., Hou, K., Zhou, T., Shi, B., Zhang, C., Zhu, L., Li, B., Wang, J., & Wang, J. (2022). Polyhalogenated carbazoles (PHCZs) induce cardiotoxicity and behavioral changes in zebrafish at early developmental stages. Science of The Total Environment, 841, 156738. https://doi.org/10.1016/j.scitotenv.2022.156738 Annotation:

This paper is very useful for the section in my literature review that focuses on the cyp1A gene.

Du et al. 2022 found that the expression of the cyp1A and cyp1B1 genes were downregulated when exposed to polyhalogenated carbazoles (PHCZs) which induced both cardiotoxicity and behavioral changes in zebrafish.

3. Ji, C., Yan, L., Chen, Y., Yue, S., Dong, Q., Chen, J., & Zhao, M. (2019). Evaluation of the developmental toxicity of 2,7-dibromocarbazole to zebrafish based on transcriptomics assay. Journal of Hazardous Materials, 368, 514–522. https://doi.org/10.1016/j.jhazmat.2019.01.079

Annotation:

I have not used this exact paper in my literature review but it is going to be useful for the section that focuses on the developmental effects of environmental contaminants on zebrafish.

4. McIntyre, J. K., Spromberg, J., Cameron, J., Incardona, J. P., Davis, J. W., & Scholz, N. L. (2023). Bioretention filtration prevents acute mortality and reduces chronic toxicity for early life stage coho salmon (Oncorhynchus kisutch) episodically exposed to urban stormwater runoff. Science of The Total Environment, 902, 165759. https://doi.org/10.1016/j.scitotenv.2023.165759

Annotation: Again, this paper is an ideal paper for overall research framework and the importance of my research project.

McIntyre et al. analyzed untreated and treated urban runoff in the early life stages of Pacific salmon and found that survival and morphological development threats were reduced greatly when runoff was properly filtered using bioretention methods (2023).

5. Nicomel, N. R., & Li, L. (2023). Review of 6PPD-quinone environmental occurrence, fate, and toxicity in stormwater. Ecocycles, 9(3), 33-46. https://doi.org/10.19040/ecocycles.v9i3.347

Annotation: This paper is a great resource for an overview of what's been studied on a very popular environmental contaminant in aquatic ecosystems. Go back to this paper for more information. 111

Another environmental contaminant that is of recent growing concern in stormwater is 6PPD-quinone (widely used to improve the durability of rubber). A review of this toxin by Nicomel and Li 2023 demonstrated that the main driver for this chemical to enter ecosystems is through stormwater runoff and therefore poses a significant threat to aquatic organisms. References:

- McIntyre, J. K., Spromberg, J., Cameron, J., Incardona, J. P., Davis, J. W., & Scholz, N. L. (2023). Bioretention filtration prevents acute mortality and reduces chronic toxicity for early life stage coho salmon (*Oncorhynchus kisutch*) episodically exposed to urban stormwater runoff. *Science of The Total Environment*, 902, 165759. <u>https://doi.org/10.1016/j.scitotenv.2023.165759</u>
- Du, B., Lofton, J. M., Peter, K. T., Gipe, A. D., James, C. A., McIntyre, J. K., Scholz, N. L., Baker, J. E., & Kolodziej, E. P. (2017). Development of suspect and non-target screening methods for detection of organic contaminants in highway runoff and fish tissue with high-resolution time-of-flight mass spectrometry. *Environmental Science: Processes & Impacts*, 19(9), 1185–1196. <u>https://doi.org/10.1039/C7EM00243B</u>
- Zhang, S.-Y., Gan, X., Shen, B., Jiang, J., Shen, H., Lei, Y., Liang, Q., Bai, C., Huang, C., Wu, W., Guo, Y., Song, Y., & Chen, J. (2023). 6PPD and its metabolite 6PPDQ induce different developmental toxicities and phenotypes in embryonic zebrafish. *Journal of Hazardous Materials*, 455, 131601. https://doi.org/10.1016/j.jhazmat.2023.131601
- Xie, S., Feng, Y., Zhou, A., Lu, Z., & JixingZou. (2023). Comparative analysis of two new zebrafish models: The cyp1a low-expression line and cyp1a knockout line under PAHs exposure. *Gene*, *869*, 147391. <u>https://doi.org/10.1016/j.gene.2023.147391</u>
- Kriech, A. J., & Osborn, L. V. (2022). Review of the impact of stormwater and leaching from pavements on the environment. *Journal of Environmental Management*, 319, 115687. https://doi.org/10.1016/j.jenvman.2022.115687
- Barbosa, A. E., Fernandes, J. N., & David, L. M. (2012). Key issues for sustainable urban stormwater management. *Water Research*, *46*(20), 6787–6798. https://doi.org/10.1016/j.watres.2012.05.029
- Du, Z., Hou, K., Zhou, T., Shi, B., Zhang, C., Zhu, L., Li, B., Wang, J., & Wang, J. (2022). Polyhalogenated carbazoles (PHCZs) induce cardiotoxicity and behavioral changes in zebrafish at early developmental stages. *Science of The Total Environment*, 841, 156738. <u>https://doi.org/10.1016/j.scitotenv.2022.156738</u>
- Padmanabhan, S. (Ed.). (2014). Handbook of Pharmacogenomics and Stratified Medicine. In *Handbook of Pharmacogenomics and Stratified Medicine* (p. i). Academic Press. <u>https://doi.org/10.1016/B978-0-12-386882-4.00047-5</u>

<sup>&</sup>lt;sup>1</sup> You are not locked into this title; we want you to identify the main point or topic of your thesis.

 $<sup>^2</sup>$  You might discuss a selection of case studies, sampling methods, experimental design, and/or specific hypotheses you will test. You should also address any specialized knowledge or skills that are necessary to complete the research.

<sup>&</sup>lt;sup>3</sup> If you are planning to use existing data, explain the specific source, contact information, arrangement with collaborating agencies, and expectations about use of data and final products of your research. If you are planning to gather new data, describe specific methods, time, place, and equipment that will be required.

<sup>&</sup>lt;sup>4</sup> If you're not sure where to start, consult a 'Code of Ethics' or other similar document from an academic society in an applicable field of study.

<sup>&</sup>lt;sup>5</sup> If you are collecting ANY samples or data, even observational data, on public lands (city, county, state and/or federal) it is your responsibility to find out the permit requirements BEFORE you collect data. Conducting research with tribal members/on tribal lands will have different and additional requirements.

<sup>&</sup>lt;sup>6</sup> Your *positionality as a researcher* refers to the fact that one's "…beliefs, values systems, and moral stances are as fundamentally present and inseparable from the research process as [one]'s physical, virtual,

or metaphorical presence when facilitating, participating and/or leading the research project..." (The Weingarten Blog 2017).