Long-term Anthropogenic Effects on Aquatic Communities and Habitat Prior to Restoration Efforts in the Flint River, Flint MI

BY

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Master's Degree Thesis

Submitted to
University of Michigan-Flint
In partial fulfillment of the requirements
for the degree of

MASTERS OF BIOLOGY

Department of Biology

2025

LONG-TERM ANTHROPOGENIC EFFECTS ON AQUATIC COMMUNITIES AND HABITAT PRIOR TO RESTORATION EFFORTS IN THE FLINT RIVER, FLINT MI

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August 22nd, 2025

ABSTRACT

LONG-TERM ANTHROPOGENIC EFFECTS ON AQUATIC COMMUNITIES AND HABITAT PRIOR TO RESTORATION EFFORTS IN THE FLINT RIVER, FLINT MI

BY

Arianna M. Elkins

The Flint River, Michigan has seen centuries of anthropogenic disturbances from industrialization and agriculture. The river has also seen alterations in the forms of dams, channelization and land use in relation to urbanization. The Flint River basin includes 7 counties in southeast Michigan. The Flint River reaches 228.8 km (142.2 miles) in length forming a 3,450 square kilometer (1,332 square mile) drainage network with its tributaries. The Flint, Cass, Shiawassee, and Tittabawassee Rivers merge to form the Saginaw River, then flow into the Saginaw Bay in Lake Huron. The combination of anthropogenic disturbances and alterations have led to long-term changes in the riverine ecosystem across the watershed. The Flint River has seen increased support by being granted a large-scale restoration effort that intended to re-naturalize the river by reducing habitat fragmentation and increasing habitat quality for organisms and human recreation. This project focuses on collecting data in the form of pre-restoration data, to provide a baseline for understanding post-restoration effects on the aquatic habitat. We sampled fishes in 2019, and 2021-2024 to calculate taxonomic and functional diversity metrics. Additionally, we employed two different side-scan sonars to assess agreement between sonars, and understand underwater substrate availability, as fishes have specific habitat preferences in terms of feeding, shelter, and reproduction. To understand contaminant levels, we collected benthic sediment, surface water, and fish tissue at 6 sites along a gradient of

anthropogenic impacts along the Flint River, Michigan to be tested for volatile organic compounds (VOCs), heavy metals, persistent organic pollutants (pesticides and industrial chemicals), and synthetic chemicals. For comparison to contaminant levels in each media, we collected macroinvertebrates and conducted habitat assessments at all chemical contamination sites. Our fish community results suggest that functional diversity of fishes is affected by the presence of Hamilton Dam. Our habitat mapping results suggest that there are current limitations to using these sonars to assess underwater substrate availability in river systems and this application requires more refinement. There is a need for comprehensive, site-specific assessments for understanding the ecological impacts of low-head dams in urban river systems. Chemical contamination collection results indicate that the Flint River is suffering from short- and long-term pollution and calls for further testing to guide restoration and cleanup efforts. Lastly, we found that macroinvertebrate collection can be a quick and cost-effective way to understand the general health of an area. The results of this study highlight the need for extensive site-specific studies prior to riverine restoration events and should raise discussion about future cleanup events on the Flint River.

DEDICATION

I dedicate this thesis to the Flint River and to the City of Flint, Michigan. Without the beauty of the river and its ability to sustain life, and without the rich spirit and resilience of Flint, I would never have discovered the deep inspiration that led me to pursue wildlife biology and complete this thesis. This place will always hold a most special place in my heart; it is where I discovered true passion.

ACKNOWLEDGMENTS

I would like to thank my graduate advisor Dr. Heather Dawson for always believing in me. Her resourcefulness in realizing the need for research on the Flint River has helped shaped me as an aspiring biologist, as well as provided this opportunity to many other students. Without her, I may have never made the switch from engineering to biology, a change that has completely changed the course of my future and my outlook on life. She recognized me as an undergraduate and allowed me to inherit a thesis project that was already years in the making; a true honor. She provided a large amount of experience, funding opportunities and traveling opportunities that I will cherish forever. She challenged me every step of the way and never doubted that I would succeed. Her trust, guidance and confidence have been pivotal in my life, and her lessons will remain for a lifetime.

I would like to thank my graduate committee members, Dr. Matthew Fhaner and Dr. Cam McLeman. This project would not have been possible without their varying expertise and guidance along the way. Dr. Fhaner was the catalyst for my time spent as an Analytical Chemist at the Flint Community Water Lab during my thesis work; an endeavor that helped shape me as not only a biologist, but a chemist as well. This opportunity allowed me to manage full time employment while completing my master's degree and will be pivotal in my future career endeavors. Dr. McLeman provided unwavering computing and statistical support as well as helped fund multiple sonar devices that were necessary for completing a well-rounded assessment for this thesis.

I would like to thank the University of Michigan-Flint, C.S Mott Foundation, Flint River Watershed Coalition, Michigan Department of Health and Human Services and the Michigan

Department of Environment, Great Lakes and Energy, for providing funding, resources, and assistance to understand the health of the Flint River.

I would like to acknowledge all the students that participated in this study and made it possible. No part of this project could be completed by one single person. Chloe Summers, Bryan Meisen, Dani Henzarek, Nicklas Smith, Justin Franks, Sydney Tremblay, Nick Weld and many others who were eager to assist whenever needed. Thank you to Bryan Meisen, who always made my visons come to life and was always ready with his fishing poles to meet our goals. Thank you to Dani Henzarek, for always providing unwavering support and understanding. A very special thanks to Chloe Summers, who pioneered the way for this project, and provided support and endless enthusiasm for our endeavors. I am extremely thankful for her support and unconditional love.

A special thanks to Cameron Bodine, the developer of PING-Mapper, and those at BioBase Maps, who went out of their way to help me navigate sonar mapping and analysis. I appreciate them taking their time to help me grasp and understand new and developing technology.

Lastly, I would like to thank my family and friends who have supported me through everything. Thank you to my mom and dad, who have always seen my potential and influenced me to seek higher education. A special thanks to my partner along the way, Trevor Tessmar, for never doubting that I would see this through and for unconditionally loving me through all my doubtfulness and challenging times (and endless studies during football games). The trust that all these people have in me was the single most important thing in providing the confidence I needed to succeed.

Table of Contents

ABSTRACT 2
DEDICATION 4
ACKNOWLEDGMENTS 5
LIST OF TABLES 9
LIST OF FIGURES 11
INTRODUCTION 15
I. Chapter 1 ..... **21**
Abstract 21
Introduction 23
Methods 26
Study Site 26
Fish Communities 27
Habitat Mapping 28
Fish Communities 29
Habitat Mapping 31
Results 32
Fish Communities 32
Habitat Mapping 34
Discussion 34
II. Chapter 2 ..... **50**
Abstract 50
Introduction 53
Methods 60
Study Area 60
Contaminants 61
Macroinvertebrates 63

Analyses of Contaminants 64
Analyses of Macroinvertebrates 65
Results 65
Contaminants 65
Sediment 66
Surface Water 67
Piscivores 68
Omnivores 69
Benthivores 69
Macroinvertebrates and Habitat Scores 69
Discussion 70
CITATIONS 97

LIST OF TABLES

Table I-1. List of how determinations were derived for ground truthing the habitat mapped with multiple different sonars. Determinations were grouped into soft, medium and hard. A visual determination was made between "muck mud" (as soft), "muck sand" (as medium), and "boulder/cobble" (as hard) for the ground truth determinations and compared to determinations made by each sonar (HumminBird Helix7 G4 unit, Lowrance HDS-7 Live) and sonar analysis programs (PING-Mapper, BioBase Maps)..... 39

Table I-2. Outcomes of model selection using the Akaike Information Criterion corrected for small sample sizes (AICc) of a mixed effects model evaluating predictors for location to the dam and sampling year. Model selection was based on AICc, and only models with $\Delta AIC < 4$ were averaged and presented in this table. Among the models evaluated, neither year nor location had a statistically significant effect on species richness or Shannon diversity index. Only location (upstream of the dam) had a statistically significant negative effect on functional dispersion as indicated by the P-value and 95% confidence intervals..... 40

Table I-3. Weighted Cohen's Kappa results as a statistical measure of agreement between BioBase Maps and the ground truth data, PING-Mapper and the ground truth data, and between the two sonars, accounting for the degree of disagreement. A weighted matrix was used that gives more credit when disagreements are close (soft vs. medium) vs. determinations that are more different (soft vs. hard). 41

Table II-1. Habitat assessment scores following the Michigan Department of Environmental Quality Procedure 51 for wadable and non-wadable streams as applicable (Michigan Department of Environmental Quality, 2013). This is a 100-point scale with scores indicating excellent (100-84), good (83-56), marginal (55-28) and poor (27-0). Macroinvertebrate scores are shown for spring and fall of 2023, and spring of 2024 following methods and scoring from the Flint River Watershed Coalition (FRWC) protocol with modifications to adapt to non-wadable rivers where applicable. The scale for this method is limitless, with scores indicating excellent (>48), good (48-34), fair (33-19) and poor (<19)..... 77

Table II-2. Of 30 models analyzed with Akaike Information Criterion corrected for small sample sizes (AICc) modeling, there were two top models that provided significance and the only models with a delta $AIC < 4$ and variance inflation factors > 6. Overall, habitat assessment score was the only variable that provided a significant positive effect on macroinvertebrate score reflected in the 95% confidence intervals. 78

Table II-3. Results of benthic sediment contamination concentrations with reference values. Reference values for Toxic Equivalency of Polychlorinated Biphenyls (TEQPCBs) was derived from the 2,3,7,8-TCDD pgTEQ/g referenced in the Canadian Sediment Quality Guidelines, 2002. The TEQPCB represents the cumulative toxicity of all PCB compounds found in each sample, as some compounds are more toxic than others. Perfluorooctanesulfonic acid (PFOS) does not have sediment quality guidelines, so no comparison can be made. Vinyl chloride and 1,4-dioxane were added as they are believed to be signature chemicals in the

2022 Lockhart Chemical spill. Reference values for vinyl chloride and 1,4-dioxane were derived from guidelines of rescinded U.S. EPA, Region 5 RCRA, Ecological Screening Levels (U.S. EPA, 2003). Values below the Method Detection Limit (MDL) were recorded as zero. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration. 79

Table II-4. Results of benthic sediment heavy metal contamination concentrations with reference values. Methylmercury (Hg), arsenic, cadmium, and lead reference values were derived from MacDonald Sediment Quality Guideline (SQG), specifically the consensus-based Probable Effects Concentrations (PEC). PEC's identify concentrations of contaminants that are above the harm threshold for sediment-dwelling organisms (Macdonald et al., 2000). Selenium values were referenced from the EGLE Water Resources Division Procedure 48. Values below the Method Detection Limit (MDL) were recorded as zero. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration. Values were used to calculate Probable Effects Concentrations (PECq) for methods outlined in MacDonald 2000 to determine if values returned were statistically higher than the provided reference value and with high likelihood to cause harm effects to organisms. (indicated by asterisk).. 80

Table II-5. Concentrations for select contaminants in surface water samples. Threshold values were obtained from the National Recommended Water Quality Criteria – Human Health Criteria Table, using the "Organism Only" category. which indicates the health of the water assuming that people are not drinking the water, but still consuming fish from it over their lifetime. These thresholds are designed for average adults based on an average body weight and height. Any contaminant concentrations above these thresholds may pose a potential health risk to people who consume fish from the affected waters due to bioaccumulation. TEQPCB is listed in parts per trillion (ppt), where the remaining are listed in parts per billion (ppb). Contaminants shown include heavy metals, and persistent organic pollutants (e.g., PCBs, PFOS). The TEQPCB threshold (0.000064 ppb) applies to total PCBs, not toxic equivalency (TEQPCB). PFOS thresholds are from the EPA's 2024 draft human health criteria; 0.0000074 ppb represents the low end of PFOS concentrations observed in surface water globally. Lead is not regulated under these water quality criteria for human health; instead, it falls under the Lead and Copper Rule for drinking water. Michigan's lead and copper rule states an action limit for lead of 12 ppb. This action level signifies that if a home has multiple points in the home consistently testing over 12 ppb, the water is deemed unsafe for normal consumption. This value is reported in this thesis for reference only, as the Lead and Copper Rule takes into consideration the normal household drinking water consumption, and does not consider the biomagnification factor present in the environment. Values below the Method Detection Limit (MDL) were recorded as zero. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration. Statistical significance above the threshold value indicates that of the replicates at that site,

greater than 10% of the measurements of water quality conditions exceed numeric reference value criteria, as stated by section 303(d) of the Clean Water Act. 81

Table II-6. Piscivore tissue contaminant concentrations from fish collected in 2024. Piscivores were tested for heavy metals due to the bio accumulative nature of the metals. Lead was removed from the figure as there were no levels of lead found above the Method Detection Limit (MDL). Levels for mammalian exposure were used for reference in this table. Values below the MDL were recorded as zero. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration. 82

Table II-7. Omnivores collected in 2024 with the added exception of rock bass were tested for perfluorooctane sulfonic acid (PFOS) and surrogates due to the nature of these contaminants and fish to reside throughout the water column. Zeros in the figure represent values that were below the method detection limit (MDL). Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration. 84

Table II-8. Benthivore tissue contaminant concentrations for fish collected in 2024. Benthivores were tested for PCBs and DDT's and derivatives due to the nature of these contaminants to settle in sediment, and the nature of these fish to reside and feed close to benthic sediment. 2,4-DDD and 2,4-DDT were removed from the figure as there were no levels found above the Method Detection Limit (MDL). Zeros in the figure represent values that were below the MDL. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration. TEQPCB indicates the Toxic Equivalency (TEQ) which is a value that represents the toxicity relative to all PCB compounds found in the sample (US EPA, 2012). RfD are not yet evaluated for DDE, DDD or PCBs. Oral Reference Doses were added for reference to data, from the USEPA Integrated Risk Information System. Oral Reference Dose is expressed in mg/kg-day and represents levels of ingestion that can cause certain toxicity effects on the body. RfD should be interpreted as consumption in ppb per day. (1 ppm = 1 mg/kg of contaminant and 1 ppb = 1 ug/kg). Mercury RfD is derived from the methylmercury source 86

LIST OF FIGURES

Figure 1. Timeline of anthropogenic influences on the Flint River and the City of Flint, Michigan, highlighting major industrial developments and population changes in the City of Flint. Key events include the channelization of the river in the late 1800s, the rise of the automobile industry in the early 1900s, peak urban population in 1960, and subsequent deindustrialization and demographic shifts. Information taken from (Dandaneau, 1996; Leonardi & Gruhn, 2001; U.S. Census Bureau, 2024). 42

Figure 2. Dams along the Flint River and its local tributaries. Nellis, Unsel, and Historical Crossroads Gristmill Dam are located on tributaries. The Hamilton Dam at the time of this

study was a low-head dam consisting of a ladder type weir as the superstructure had been removed in 2018. The Hamilton Dam has no effective fish passage upstream and is the first barrier of the Flint River to potamodromous fishes. Utah Dam no longer impedes flow of fish movement. Mott Dam has no effective fish passage, while Holloway Dam fish passage is restricted to the sluice gates or downstream movement over the spillway. Fabri dam is not considered a dam that prevents fish passage or withholds large amounts of water and therefore was not included in the EGLE dam dataset used for this map. The water flows southwest from the Holloway Dam to the Hamilton Dam and begins flowing northwest, eventually flowing into the Shiawassee River, then the Saginaw River, and later into the Saginaw Bay in Lake Huron. Data from ArcGIS online data (Environment, Great Lakes, and Energy, 2023a) and basemap data from Esri 2012..... 43

Figure I-1. Location of the Flint River and its watershed (gray), with the City of Flint indicated. The Flint (blue), Cass (red), Shiawassee (yellow), and Tittabawassee (green) Rivers merge to form the Saginaw River (white), which ultimately flows into the Saginaw Bay in Lake Huron. Map created in ArcGIS Pro (Esri, 2009). 44

Figure I-2. Google Map aerial view of study sites on surrounding the Hamilton Dam on University of Michigan-Flint Campus, Flint Michigan. Sample sites 1-4 edited using the Paint application. Sites 1 and 3 are downstream of the Hamilton Dam. Sites 4 and 2 are upstream of the Hamilton Dam. Figure created by Chloe Summers and used with her permission. 45

Figure I-3. Google Earth imagery of the location mapped for habitat mapping, and the location of ground truth sampling on the Flint River, Flint Michigan. The habitat mapping covered the entire restoration area, extending 2590 river meters upstream of the Hamilton dam (green point), and 820 meters downstream of the dam (blue point). The Hamilton Dam is indicated in yellow. The ground truth sampling points are distributed along the red river segment, extending 1500 river meters upstream of the dam. Image created in Google Earth. 46

Figure I- 4. Raw data for fish caught in 2023 and 2024 upstream and downstream of Hamilton Dam, Flint Michigan. Species richness is the total number of species found in an area, where Shannon diversity index quantifies species diversity while considering both species richness and evenness of species abundances; with higher values indicating greater diversity. Functional dispersion is a measure of how functionally diverse fish species are in terms of ecological roles by measuring the degree of trait difference among species..... 47

Figure I- 5. Visualization of differences in taxonomic diversity results between sites using Non-metric multidimensional scaling (NMDS) ordinations. The ellipses are ordination axes summarizing variation in trait composition. The overlapping ellipses indicate that taxonomic communities were not different above vs. below the dam for combined data from 2019, and 2021-2024. Permutational Multivariate Analysis of Variance (PERMANOVA) results = ($F = 0.658$, $R^2 = 0.19791$, $p = 0.919$). 48

Figure I- 6. Visualization of differences in functional diversity results between sites using Non-metric multidimensional scaling (NMDS) ordinations. The ellipses are ordination axes

summarizing variation in trait composition. The non-overlapping ellipses indicate that functional communities were different above vs. below the dam for combined data from 2019, and 2021-2024. Permutational Multivariate Analysis of Variance (PERMANOVA) results = ($F = 8.6235$, $R^2 = 0.7638$, $p = 0.002$). 49

Figure II- 1. Map of known perfluoroalkyl substances (PFAS) presence sites, the chemical contamination collection sites, and location of the 2022 Lockhart Chemical spill in the Flint River Watershed Basin. Fish consumption guidelines are based off previous testing of fish tissues, the amount of safe consumption can vary depending on the weight and age of the person (US Food and Drug Administration, 2024)..... 88

Figure II- 2. Chemical contamination collection sites along a gradient of anthropogenic disturbances along the Flint River, Michigan. Location of 2022 Lockhart Chemical spill and location of the building are included. The Hamilton Dam is located at 3/4. Dam down/upstream. The Holloway Dam is located ~700 river meters above the 5. Urban upstream site. 89

Figure II- 3. There was a positive relationship between macroinvertebrate collection scores following FRWC methods and scoring, and habitat assessments conducted following EGLE habitat assessment methods and scoring..... 90

Figure II- 4. Spatial distribution of key contaminant findings across six study sites along the Flint River, ordered from upstream (right) downstream (left), following the direction of river flow. Sites include non-urban downstream (Flushing Township Nature Park), urban downstream (Mott Park), directly upstream and downstream of Hamilton Dam (3/4), urban upstream (Stepping Stone Falls), and non-urban upstream site (Irish Road Fishing Site). The visualization highlights spatial patterns in contaminant occurrence, including elevated PCBs, mercury, cadmium, arsenic, and lead in specific compartments for each site. For site 5. Urban upstream there were no negative conclusions to be gathered. In fact, this site had the lowest mean levels of metals in fish tissue..... 91

Figure II- 5. Relative effects graphs of sediment contaminants tested. Sediment samples were taken in duplicates at each of the 6 sites. Only contaminants with results are shown. Adjustments were made to remove contaminants with high multicollinearity from analyses (VIF > 6; 1,4-dioxane, vinyl chloride, DDx). The remaining variables in the model were significantly different between each other and between sites ($P < 0.05$). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites. 92

Figure II- 6. Relative effects graphs of surface water contaminants tested. Surface water samples were taken in duplicates at each of the 6 sites. Only contaminants with results are shown. Adjustments were made to remove contaminants with high multicollinearity from analyses (VIF > 6; 1,4-dioxane, vinyl chloride, DDx, mercury, cadmium, selenium). The remaining variables in the model were significantly different between each other and between

sites (P =0.003). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites. 93

Figure II- 7. Relative effect graph for piscivore contaminants tested in 2024 and for fish collected at sites 3 and 4, prior to the toxic waste spills. Piscivores were tested for As, Hg, Se, Pb, and Cd. Only contaminants with results are shown. Adjustments were made to remove contaminants with high multicollinearity from analyses (cadmium and lead). The remaining variables in the model were significantly different between each other and between sites (P < .05). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites. 94

Figure II- 8. Relative effect graph for omnivore contaminants tested in 2024 and for fish collected at sites 3 and 4, prior to the toxic waste spills. Omnivores (with the addition of rock bass) were tested for PFOS only. Statistical analysis reveals differences between the sites in contaminant levels (P < 0.001). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites. 95

Figure II- 9. Relative effect graph for benthivore contaminants tested in 2024 and for fish collected at sites 3 and 4, prior to the toxic waste spills. Benthivores were tested for the DDTs and derivatives and PCBs. Adjustments were made to remove contaminants with high multicollinearity from analyses (DDEs, 2,4-DDT). Statistical analysis reveals no differences between the sites in contaminant levels (P > 0.05). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites. 96

INTRODUCTION

Once a lifeline for industry and trade, the Flint River in Flint, Michigan has endured more than a century of environmental abuse (**Figure 1**). The Flint River is a tributary to the Saginaw River system, therefore a tributary of Lake Huron. This study focuses on the effects of anthropogenic stressors that have all simultaneously changed the Flint River ecosystem over many years such as the presence of a major dam (Hamilton Dam), other physical river alterations, and short- and long-term pollution of the Flint River and its inhabitants.

The mainstem of the Flint River has 4 major dams (including Hamilton Dam): Utah Dam, C.S Mott Dam, and Earl Holloway Dam were constructed in 1928, 1971, and 1973, respectively (**Figure 2).** The original Hamilton Dam, creating the Hamilton impoundment, was constructed in the present downtown Flint for aiding in the logging industry (Stockrahm, 2022), later to be replaced by a concrete dam with a storage capacity of 100 acre-feet in 1920. The Utah Dam is no longer operational, not impeding fish movement as the gates are permanently locked open. C.S Mott Dam and Earl Holloway Dam create the C.S Mott Lake and Holloway Reservoir impoundments, respectively. A much smaller inflatable dam, Fabri Dam, was put in place just downstream of Hamilton Dam in 1979 for recreation and visual enhancement (Genesee County Parks, n.d.-c). This dam did not have the capacity to create an impoundment, and only at abnormally low water levels was this dam believed to have the capability to prevent fish movement upstream. All impoundments suffer from increases in sediment and nutrient loading, although they do provide recreation and angling opportunities (Leonardi & Gruhn, 2001).

Throughout the duration of this study, the downstream reach of the Flint River aquatic habitat was fragmented by two small dams: Hamilton Dam and Fabri Dam. Small dams have less danger in terms of failing and structural degradation, but for this reason they lack attention and

tend to be older than large dams and in poorer condition (Poff & Hart, 2002). With the condition of Hamilton Dam, and having no remaining economic use to the city, this century-old dam received notice and was considered a high hazard to humans by the Michigan Department of Natural Resources (Stockrahm, 2022).

The superstructure of Hamilton and Fabri dams, the portion of the dams that are located above the water level, were removed in 2018 (Genesee County Parks, n.d.-a). With the absence of a superstructure, what remained of the dams were then considered to be low-head dams with a ladder-type weir, an underwater cement structure that runs perpendicular to the flow of water. Low-head dams often do not provide hydroelectricity or have great capacity to control floods (Poff & Hart, 2002). The Fabri Dam weir prevented fish passage upstream only at low water levels often seen in late spring and summer, however the Hamilton Dam weir prevented fish passage upstream no matter the water levels, thus the Hamilton Dam has historically prevented fish passage upstream for the entirety of its lifetime.

Funding was secured from multiple agencies and organizations to implement the complete removal of dams and restoration of the Flint River. The restoration event was completed in 5 phases and consisted of the removal of the Hamilton and Fabri Dams, the addition of 6 rock rapid riffles and beautification for human recreation along the Flint River trail. The restoration even added rock rapid riffles with intent to facilitate fish movement upstream by providing resting spots for species not able to withstand high water velocity for extended periods of time. The phases of the restoration event were completed in early 2025 and reconnected approximately 40 kilometers (25 miles) of river with the restoration event including recreational and environmental advantages (Stockrahm, 2022). The effects of this restoration may impact more than the Flint River as the Flint River is a tributary to the Saginaw River system and this

system and the bay it empties into in Lake Huron are a Great Lakes Area of Concern (AOC) with degradation of benthos, fish and wildlife populations a major concern (United States Environmental Protection Agency, n.d.-c).

In addition to habitat fragmentation caused by structures and dams on the Flint River, the Flint River has suffered from the anthropogenic effects of industrialization in the 19th to mid-20th century, and effects of deindustrialization beginning in the 1970s (**Figure 1**). In 1960 Flint's population reached nearly 200,000 people (**Figure 1**), the population increase widely attributed to the booming automobile industry in Flint during that time. The deindustrialization of Flint began in the 1970's, when General Motors began relocating local automobile manufacturing factories outside of Flint, Michigan. In present day, Flint remains the largest city in Genesee County with a population of 80,000 people, but the highest unemployment rate in the county (Data Commons, n.d.; U.S. Census Bureau, 2024). Not only did this change to the city bring a decline in workforce and population but left behind remnants of legacy contaminants from car makers and other industries, which will be discussed in greater detail in Chapter 2.

Anthropogenic disturbances are the main forces affecting our waterways globally (Fergus et al., 2023; Ji et al., 2021; Paul & Meyer, 2001). Urban presence brings significant alterations to the environment and surrounding riverine habitats, manifesting in diverse ways such as changes in pollution, channelization, impervious surface cover, and structural developments, all of which contribute to riverine abiotic and biotic changes. Structures such as roads, railroads, county drains, and bridge crossings act as impervious surfaces, which can further fragment habitats, exacerbate erosion, alter water chemistry, and disrupt river habitats and consequently fish community structure (Leonardi & Gruhn, 2001). Historically, the river has seen much use for

transportation of goods, dumping and diluting of industrial and human waste and non-point source pollution from runoff of growing agricultural and urban developments.

Following the April 1947 flood that caused $4 million in flood damage within the City of Flint (Wiitala & Krieger. R, 1963), a flood control project was conducted by the United States Army Corps of Engineers in the City of Flint in 1963. This project led to large changes in the river by complete channelization of one mile of the Flint River in the City of Flint, leading to concrete lining of the riverbed and banks, impacting the riverbed and riparian habitat in the process (Leonardi & Gruhn, 2001). Currently, this one mile of the Flint River remains channelized. The channelization of river segments contributes to a decline in wetlands and their water filtration capacity, exacerbating erosion, impervious surface area, warmer water temperatures, and sedimentation, thereby compromising water quality, flow stability, organismal productivity, and diversity (Leonardi & Gruhn, 2001; Talmage et al., 1999). Riparian corridors are crucial for providing habitat, nutrients, and woody debris to riverine ecosystems, and undergo degradation due to alterations in the natural flow of the river caused by channelization (Leonardi & Gruhn, 2001; Poff & Hart, 2002; Poff et al., 1997). This decrease in the natural filter capacity of wetlands promotes non-point source pollution from agricultural fields, construction sites, impervious surfaces, railroads, and septic sewage; historically the greatest deterioration factor of the water quality of the watershed (Leonardi & Gruhn, 2001). Erosion leads to an increase in sedimentation in the water system affecting the sediment size variability and availability. Sedimentation, the accumulation of nutrient-rich fine sediments surrounding a dam, is inevitable (Riggsbee et al., 2005). Sedimentation is when nutrient-rich fine sediments accumulate in the waterways and upstream of dams (Riggsbee et al., 2005) allowing sediment free water to pass over the dam. Sediment-free water can be troublesome as it has a high ability to hold sediment

and therefore increased erosive power, increasing sediment movement downstream. These effects can lead to a suffocation of eggs, larvae, and less mobile organisms; contribute to habitat homogeneity; change the habitat of the river, morphology of the channel, and structure of riparian and aquatic species (Leonardi & Gruhn, 2001; Poff & Hart, 2002; Poff et al., 1997). Erosion is a stand-alone problem, as the worlds' soil is being lost 13-40 times faster than the natural rate of renewal (Zuazo & Pleguezuelo, 2008). Overall, these anthropogenic influences pose significant challenges to maintaining healthy fish habitats and biotic biodiversity within riverine ecosystems.

Impervious surfaces exacerbate water temperatures and facilitate runoff into water bodies, which, coupled with population density, positively correlates with increased water temperatures, sodium, and chloride ion concentrations in water bodies and negatively correlates with aquatic species richness and diversity in urban settings (Talmage et al., 1999). This can be problematic as the Flint River watershed is already a warm water temperature regime, although there can be some heterogeneity in water temperatures as areas with undisturbed landscapes and an amount of groundwater inflow can harbor cooler water temperatures in less urban areas (Leonardi & Gruhn, 2001) These factors collectively contribute to habitat alteration through urban water runoff, elevated water temperatures, erosion, loss of riparian corridors, and modifications in water chemistry.

This project aimed to evaluate ecosystem health metrics across a gradient of anthropogenic disturbances on the Flint River by providing a comprehensive ecological assessment of the river prior to the restoration event. To understand how these simultaneously working anthropogenic disturbances have altered the water quality, fish and macroinvertebrate populations, and habitat integrity of the Flint River over time we collected data for a total of 5

years prior to the dam removal restoration efforts. To understand fish assemblages, we used 4 methods of fish collection at 2 locations directly surrounding Hamilton Dam (Chapter 1) and widened our scope to 4 additional sites along the Flint River with varying anthropogenic impacts and surrounding land use (Chapter 2). To understand water quality, we collected macroinvertebrates at all 6 sites to assess species assemblages and compare scores to habitat assessments. We also collected fish, benthic sediment, and river water which were tested at an outside lab for volatile organic compounds (VOCs), heavy metals, persistent organic pollutants (pesticides and industrial chemicals), and synthetic chemicals. Reference values were used for contamination analysis to understand if the present contaminant levels are above safe environmental thresholds for humans and fish.

I. Chapter 1

FISH COMMUNITY DIVERSITY AND IN-STREAM HABITAT ABOVE AND BELOW THE HAMILTON DAM ON THE FLINT RIVER, FLINT MICHIGAN

Abstract

This study evaluated the effects of a low-head dam on fish community composition and instream habitat within a highly urbanized section of the Flint River, Michigan. The objectives of this chapter were to; 1) understand how fish assemblages compare upstream vs. downstream of Hamilton Dam, and over time; and 2) determine the best way to measure river bottom habitat availability to evaluate how habitat changes affect fish communities' post-restoration. We sampled fishes in 2019, and 2021-2024 to calculate taxonomic and functional diversity metrics. Additionally, we employed two different side-scan sonars to assess agreement between sonars, and understand underwater substrate availability, as fishes have specific habitat preferences in terms of feeding, shelter, and reproduction. We assessed both taxonomic and functional diversity upstream and downstream of Hamilton Dam over all sampling years. Catch per unit effort (CPUE) was standardized using multi-gear mean standardization to provide comparisons without gear bias. Taxonomic diversity was measured using species richness and the Shannon diversity index, while functional diversity was measured using Functional Dispersion (FDis) based on morphological and ecological traits. We applied linear mixed-effects models (LMEs) to determine the influence of location and sampling year on taxonomic and functional diversity metrics and used non-metric multidimensional scaling (NMDS) ordinations with Permutational Multivariate Analysis of Variance (PERMANOVA) to examine community composition. Functional diversity was significantly different in the upstream vs. downstream of dam locations,

while taxonomic diversity remained similar between locations, indicating that the dam may alter functional roles present more than species identity. Additionally, we tested the reliability of two sonars and two sonar-based habitat mapping tools (PING-Mapper and BioBase Maps) against ground-truthed sediment samples. While BioBase Maps showed moderately statistically significant agreement with ground truth data, PING-Mapper did not, and there was no agreement between the two sonar systems. Our results highlight the sensitivity of functional diversity to habitat modifications, the limitations of current habitat mapping methods on riverine systems, and the importance of long-term, site-specific studies in guiding dam removal and river restoration efforts.

Introduction

As society recognizes the need for watershed management and the restoration of riverine ecosystems, support for dam removals is growing among scientists and the public as a form of ecological restoration (AmericanRivers.org, n.d-b.; Babbitt, 2002). A growing number of dams now require repair or removal as they reach the end of their intended lifespan, the repair of the dam often proving to be much more expensive than removal (see Manchester Dam, Meyer Dam. Bell Park Dam, Randall Dam, Gray Reservoir Dam, Deerskin Dam: American Rivers.org, n.d.-a). The dams in this study are considered low-head dams, which are typically less than 4 meters in height, also known as weirs, and are defined as river barriers that span perpendicular to the flow of the river, with intent to manage the waterflow by raising the level of water upstream (Genesee County Parks, n.d.-c; Gillette et al., 2005). Although dam removals are increasing, researchers have not kept pace with the growing number of removals in published studies (Bellmore et al., 2017). The removal of low-head dams is an understudied area of ecology, as they represent 28% of the documented dam removals in the United States, but only comprise 12% of the published studies (Bellmore et al., 2017).

Although researchers have studied low-head dam removals in the United States, there is still a need for long-term studies surrounding dam removals that track the long-term outcomes of these removals (Babbitt, 2002; Bellmore et al., 2017; Hart et al., 2002). Most existing research focuses on short-term biological responses following dam removal, often lacks pre-removal data and rarely provides a comprehensive ecological assessment. This research gap is especially pronounced in urban settings, where river systems are further constrained by altered hydrology, legacy contaminants, and ongoing anthropogenic stressors. This enhances the need for long-term pre- and post-removal research efforts in urban river systems, where restoration impacts may differ from more pristine environments. Long-term pre-removal data can also add to the mass of

conflicting views on the ecological effects of dam removal. Furthermore, studies that analyze fish assemblages on a functional level are also limited (Smith et al., 2017). There are a plethora of studies that support the need for dam removals for supporting species richness and diversity, allowing natural flow of water and nutrients, and mitigating habitat fragmentation (Burroughs et al., 2010; Haworth & Bestgen, 2024). However, some studies suggest that dam removal does not always lead to immediate or significant ecological improvements. In some cases, the expected benefits such as increased species richness may not occur, and other factors like invasive species containment, recreational use, or flood control may be arguments for keeping the dam in place (Chick et al., 2006; Lavis et al., 2003). These conflicting findings reinforce the need for long-term studies that have strong pre-removal quantitative data that can be replicated post-restoration.

Fishes have specific habitat preferences in terms of feeding, shelter, and reproduction. The composition of fish communities is closely tied to habitat availability and quality, as diverse habitats support a greater range of ecological niches. An area with more heterogeneous habitat available to fish will show a more diverse fish community (Ferrari et al., 2018). Fishes have water depth preferences, and we can make associations between depth and the species we could expect to see in that depth (Gaston et al., 2012). Additionally, fish have substrate preferences for feeding and breeding. Classifying substrate simply as hard bottom, gravel, sand, silt, or fine-clay can add to the knowledge of the habitat available to fish species (Rudolfsen et al., 2021).

We employed two different sonars to understand underwater substrate availability. These are boat mounted side and down imaging systems that allow one to survey streams, rivers, or lakes to understand underwater habitat of navigable water bodies. These devices produce high resolution imagery providing information on depth, substrate, and large woody debris (Kaeser &

Litts, 2010). Software is necessary to process the data obtained by these sonars. The Helix7 G4 unit sonar data can be processed by a free, open-source Python language software: PING-Mapper. The HDS-7 Live unit sonar data can be processed by a paid subscription service software: BioBase Maps.

As dams alter the hydrology of the river, this allows for nutrient-rich fine sediments to accumulate upstream of the dam, this is known as sedimentation (Riggsbee et al., 2005). Sedimentation affects the sediment size variability and availability and therefore can impact the habitat of the river, morphology of the channel, and structure of riparian and aquatic species (Poff et al., 1997; Poff & Hart, 2002). Removing dams paves the way for the habitat to move from homogeneous to having a more natural substrate diversity, which will increase the prevalence and evenness of fishes in guilds (Fencl et al., 2017; Jones et al., 2023). Habitat change will affect the fishes present, but the absence of a barrier induces changes as well. Following removals of dams, the upstream reach has greater potential to see species migrating and inhabiting areas they otherwise could not (Burroughs et al., 2010; Hart et al., 2002).

Fish populations are profoundly impacted by anthropogenic disturbances, with alterations in habitat conditions such as those produced by dams triggering significant changes in fish community structure (Ferrari et al., 2018; Leonardi & Gruhn, 2001). Fishes naturally and necessarily migrate for refuge, spawning and feeding. Modifications in water temperature, nutrient flow, water chemistry, instream habitat and bottom substrate diversity directly influence the habitat available to fish, consequently leading to shifts in species richness and diversity (Hastings et al., 2016b; Leonardi & Gruhn, 2001; Talmage et al., 1999). We have seen a loss in abundance of certain species, as development on the Flint River Watershed tends to favor silt tolerant species, pollution tolerant species, and generalist species (Leonardi & Gruhn, 2001).

There are five species currently extirpated from the Flint River that have all historically used the river for spawning: lake sturgeon (*Acipenser fulvescens*), lake trout (*Salvelinus namaycush*), lake herring (*Coregonus artedi*), lake whitefish (*Coregonus clupeaformis*), and muskellunge (*Esox masquinongy*). With 77 fish species known in the watershed, 35 total species have been caught above and below Hamilton Dam since 2019 (C. Summers, University of Michigan-Flint, unpublished data), with no observed presence of these currently extirpated species. The objectives of this chapter were to; 1) understand how fish assemblages compare upstream vs. downstream of Hamilton Dam, and over time; and 2) determine the best way to measure river bottom habitat availability to evaluate how habitat changes affect fish communities' post-restoration.

Methods

Study Site

The Flint River watershed includes 7 counties in southeast Michigan. The Flint River reaches 228.8 km (142.2 miles) in length forming a 3,450 square kilometer (1,332 square mile) drainage network with its tributaries. The Flint River is the second largest tributary of the Saginaw River (Wiitala & Krieger. R, 1963). The Flint, Cass, Shiawassee, and Tittabawassee Rivers merge to form the Saginaw River, then flow into the Saginaw Bay in Lake Huron (**Figure I-1**). The mainstem of the Flint River flows through the University of Michigan- Flint campus and downtown Flint, which is where our study sites for this chapter were located. This area of the Flint River has multiple barriers in place: Hamilton, Utah, Mott, and Holloway Dams (**Figure 2**). The Hamilton Dam has historically prevented fish passage upstream for the entirety of its existence and is the first barrier blocking movement of fish upstream from the Saginaw Bay in Lake Huron (Genesee County Parks, n.d.-b). To address our objective to understand how

fish assemblages compare upstream vs. downstream of Hamilton Dam, and over time, we collected fish in the Flint River 100 meters directly upstream and downstream of Hamilton Dam (**Figure I-2**). Study sites were divided by sides of the river, alternating sides every week; we fished sites 1 and 2, and sites 3 and 4 the following week. Upstream sites (2 and 4) were more lentic in flow but contained a small riparian edge. Downstream sites (1 and 3) were more lotic in flow and contained no riparian edge; downstream sites were completely constrained by cement edges. To address our objective to determine the best way to measure river bottom habitat availability to evaluate how habitat changes affect fish communities post-restoration, we chose to map habitat covering the entire future restoration area: extending 2590 river meters upstream of Hamilton Dam, and 820 meters downstream of this dam (**Figure I-3**). We chose to ground truth the area 1500 river meters upstream of the Hamilton Dam. The zone of the river restoration extended from Utah Dam to the road crossing at Grand Traverse Street (**Figure I-3**) and the restoration was to include the removal of the Hamilton Dam and construction of six riffles (shallow area created by specific placement of rocks, with fast flowing water). The study area is a highly developed stretch of river, groundwater inflow is low, and water temperatures are in the higher range (Leonardi & Gruhn, 2001).

Fish Communities

This study is part of a larger study that has collected fish above and below Hamilton Dam since 2019, except for 2020 due to COVID-19. Fish collection for this study occurred weekly from May-August 2023, and March-June 2024, alternating sides of the river each week. We completed a total of 23 fish sampling days in 2023, and a total of 14 fish sampling days in 2024. We recorded data on rite-in-the-rain paper, scanned to pdf, and entered the data into a Microsoft Access database. We used fish collection data from all years (2019, 2021-2024) for analysis.

Fish were caught upstream by setting hoop traps for a maximum of 24 hours, backpack electrofishing in wadable areas, and hook and line. Fish were caught downstream by setting hoop traps for a maximum of 24 hours, hook and line, and cast netting. The following fish metrics were processed upon fish collection: species identification, site number, weight (kg), length (cm), and gear type used. If applicable, we recorded Floy tag number, recapture status, recaptured tag number, mortality and/or preservation number if fish were sacrificed and preserved for chemical analysis testing. If fish were greater than 16 cm, or 14 cm for sunfish, the organisms were externally tagged with a Floy tag gun. These fish were also internally tagged with 12 mm Passive Integrated Transponder (PIT) tags and their PIT tag number recorded. These PIT tags will last for the life of the fish unless somehow extruded by the fish and can be scanned by any PIT tag scanner. After capture and until processed, fish were kept in a cooler filled with river water that was aerated with a multi-hose aerator powered by a portable rechargeable battery. Fish were returned to the place of capture immediately following processing. Effort was determined by the number of cast nets thrown, hook and line minutes, hoop traps set per hour, and electrofishing minutes required to fish each study site. Effort was standardized following the approach outlined in Gibson-Reinemer et al., (2017), using catch-per-unit-effort (CPUE) for each species per gear type, averaged across methods.

Habitat Mapping

We employed a HumminBird Helix7 G4 unit (Humminbird, Racine, WI) and a Lowrance HDS-7 Live unit (Lowrance, Tulsa, OK) on a 12 ft plastic Jon boat (Sun Dolphin, Muskegon, MI). Sonar data was collected by traveling downstream, with the flow of water. Each mapped river segment was covered with 2-3 sonar tracks spaced evenly across the channel width, ensuring full coverage.

We equipped sonars on our Jon boat, modifying the installation for the sonars by mounting the sonar units to the front of the boat using a bracket and hinge assembly that preserved unit integrity. Sonar files were extracted from the Humminbird Helix 7 unit and input into PING-Mapper to receive mosaics of bottom substrate classification and later georeferenced in ArcGIS Pro using the WGS84 coordinate system. Sonar files were extracted from the Lowrance HDS7 unit and input into BioBase Maps, with no further georeferencing needed.

The habitat area mapped by the sonars required ground truthing, which determines whether habitat indicated by the sonars matches the actual habitat at several locations. To *ground truth* the habitat area mapped by the sonars, 1500 river meters were measured out from a buoy just upstream of Hamilton Dam. The area on the river where ground truthing took place was limited due to ongoing restoration construction. We randomly selected 30 points along the 1500 river meter stretch based on feasibility within construction limits and to achieve representative coverage of the area. We took our Jon boat to each point and collected a layer of underwater substrate (top 2 inches of benthic sediment) with a shovel. A visual determination was made, a picture of the sample was taken for additional confirmation, and coordinates were taken directly above the shoveled sample. A visual determination was made between “muck mud” (as soft), “muck sand” (as medium), and “boulder/cobble” (as hard: **Table I-1**). We visually analyzed results from PING-Mapper and BioBase Maps to classify the hardness determination (soft, medium or hard) from the sonars, at the points of ground truth samples. We only included points that had both sonar data and ground-truth samples in the habitat mapping analysis.

Fish Communities

We conducted all analyses in R version 4.4.3 (*R: The R Project for Statistical Computing*, n.d.) and included all years of fish collection in analyses: 2019, 2021-2024. To standardize catch

per unit effort (CPUE) across multiple sampling gears, we applied multi-gear mean standardization (MGMS) following the method described by Gibson-Reinemer et al., (2017), generating gear-independent measures of relative abundance for each fish species. Taxonomic diversity was assessed using two metrics: species richness (the total number of species per site) and the Shannon diversity index, which incorporates both species richness and the evenness of species abundances. These metrics were calculated using the vegan package in R Studio (Oksanen et al., 2025a). Functional diversity was quantified using Functional Dispersion (FDis), a measure of how functionally diverse fish species are in terms of ecological roles by measuring the degree of trait difference among species. We created community matrices for both taxonomic and functional traits using standardized CPUE values across all site-year combinations. Trait distance matrices were calculated using Gower distance gowdis, and FDis was computed using the fdisp function from the FD package (Laliberte et al., 2009; Laliberte & Legendre, 2010). Functional characteristics for each sampled species were sourced from existing trait databases (Eakins, 2025; *FishBase*, n.d.) and included trophic group, feeding and reproductive guild, fecundity, migration type, water flow preference, pollution tolerance, vertical position in water column, and the morphological traits such as maximum species length, body length to depth ratio, and caudal peduncle height to body length ratio.

We explored patterns in taxonomic and functional composition across sites with non-metric multidimensional scaling (NMDS) ordinations using the Bray–Curtis dissimilarity coefficient. Separate NMDS analyses for taxonomic (species abundance) and functional (trait-based) community data were conducted to examine differences in community structure. These ordinations were visualized using the vegan functions: metaMDS and ordiplot. In conjunction with the NMDS ordinations, differences in community composition across sites were statistically

tested using Permutational Multivariate Analysis of Variance (PERMANOVA) via the adonis2 function in the vegan package (Oksanen et al., 2025b). We applied this method to both taxonomic and functional datasets to assess whether fish assemblages or trait compositions differed significantly upstream vs. downstream of Hamilton Dam.

To assess how dominant trait values in fish communities varied by location and over time, Community Weighted Means (CWMs) were modeled as a function of dam location and year. To examine how fish diversity metrics varied by location and year, we fit linear mixed-effects models using the lme function from the nlme package (Pinheiro & Bates, 2025). We constructed separate models for species richness, Shannon diversity index, and Functional Dispersion, with location (upstream vs. downstream) and year as fixed effects, and site as a random effect to account for repeated measurements. The model structure was: Taxonomic or functional metrics ~ Location + Year + (1 | Site). We used the dredge function from the MuMIn package to perform model selection via Akaike Information Criterion corrected for small sample size (AICc; Bartoń, 2025). To avoid multicollinearity among predictors, we excluded variables with variance inflation factor (VIF) > 6. Only models with ΔAICc < 4 were averaged. This allowed us to evaluate whether trait dominance shifted upstream versus downstream of the dam, and whether these patterns changed across years.

Habitat Mapping

We completed a Cochran's Q statistical analysis (Kassambara, n.d.) to assess the agreement between sonar-based substrate classifications and ground truth samples. This non-parametric test is used to determine whether there are significant differences between two paired proportions (in this case, sonars) on a dichotomous dependent variable (0= sonar does not match to ground truth, 1=sonar does match to ground truth). The ground truth is considered the true

assessment and is the independent variable, and the test assessed if the proportion of successes measured between the sonar and ground truth is the same between sonars. We followed this analysis with a Weighted Cohen's Kappa. Weighted Cohen's Kappa is a statistical measure of agreement between two raters (or methods), accounting for the degree of disagreement. This tells us how consistently two methods classify the same thing, with a weighted matrix that gives more credit when disagreements are close (soft vs. medium instead of soft vs. hard). A Weighted Cohen's Kappa value of 0-0.20 indicates slight agreement, 0.21-0.40 indicates fair agreement, 0.41 to 0.60 indicates moderate agreement, 0.61-0.80 indicates substantial agreement, and 0.81-1.00 indicates near perfect agreement (Bobbitt, 2022). We performed a Weighted Cohen's Kappa test to compare PING-Mapper and BioBase Maps data, PING-Mapper and ground truth data, and BioBase Maps and ground truth data.

Results

Fish Communities

In 2023 we collected a total of 365 fish directly upstream and downstream of the Hamilton Dam, for a total of 23 days (5/1/2023-8/14/2023). In 2024 we collected a total of 165 fish across 14 sampling days (3/25/2024-6/10/2024). Sampling start and end dates were dependent on the weather and the anticipation of fish migration seasons. Fishing days at the dam in 2024 were more limited than 2023 due to the addition of 4 new sites and chemical contamination sample collection outlined in Chapter 2. A total of 26 fish species were recorded in 2023 and 21 in 2024, representing a subset of the 35 species historically sampled since 2019.

All taxonomic and functional diversity metrics were consistently lower upstream of the dam compared to downstream across both years sampled for this study. Values for species richness, Shannon diversity index, and Functional Dispersion declined in 2024 relative to 2023

likely due to the decreased number of sampling days in 2024 compared to 2023 (**Figure I-4**). CPUE was an additional measure used to compare fish populations upstream and downstream of the dam, across collection methods. Above the dam, rock bass (*Ambloplites rupestris*) had the highest CPUE at 0.59 in 2023-24. Downstream of the dam, smallmouth bass (*Micropterus dolomieu*) had the highest CPUE at 1.28 in 2023-24. All other species had a CPUE of under 1.

During model evaluation, we included all years of fish collection in the analyses: 2019, 2021-2024, and among the models evaluated, neither year nor location had a statistically significant effect on species richness or Shannon diversity index. The only predictor variable with a clearly supported relationship—based on model-averaged 95% confidence intervals—was upstream location of the dam ($\beta = -0.071$, 95% CI: –0.114 to –0.0305, $p < 0.05$) which had an overall negative effect on functional dispersion (**Table I-2**). This indicates that the presence of the Hamilton Dam did not have a significant effect on taxonomic diversity, however it did have a significant effect on functional diversity of our fish population.

Non-metric multidimensional scaling (NMDS) ordinations with Permutational Multivariate Analysis of Variance (PERMANOVA) showed similar results. PERMANOVA results for taxonomic diversity NMDS ordinations showed overlapping ellipses indicating there was no significant difference in taxonomic communities above and below the dam ($F = 0.658$, $R^2 = 0.19791$, $p = 0.919$). PERMANOVA results for functional diversity NMDS ordinations showed non-overlapping ellipses indicating there was a significant difference in functional communities above and below the dam ($F = 8.6235$, $R^2 = 0.7638$, $p = 0.002$). These results indicate that taxonomic communities were not significantly different between upstream and downstream sites but did show that functional diversity communities were significantly different between upstream and downstream sites (**Figure I-5; Figure I-6**).

Habitat Mapping

Cochran's Q test revealed no significant difference in the success rates of substrate classification between the sonars and the ground truth ($Q = 1.6667$, $df = 1$, $p = 0.1967$). To further assess agreement, Weighted Cohen's Kappa values were calculated. The results indicate that BioBase Maps demonstrated a statistically significant level of agreement with the ground truth beyond chance. The Weighted Cohen's Kappa between BioBase Maps and the ground truth was 0.2727 (ASE = 0.1223, Z = 2.23, p = 0.02575), with a 95% confidence interval ranging from 0.033 to 0.5124. In contrast, the Weighted Cohen's Kappa between PING-Mapper and BioBase Maps was 0.0000 (ASE = 0.08909, Z = 0.0000, p = 1.0000), with a 95% confidence interval from -0.1746 to 0.1746, indicating no agreement beyond chance between the two sonar classification methods. Similarly, PING-Mapper showed no significant agreement with the ground truth. The Weighted Cohen's Kappa between PING-Mapper and the ground truth was 0.0646 (ASE = 0.09616, Z = 0.2751, p = 0.7832), with a 95% confidence interval ranging from 0.162 to 0.2149 (**Table I-3**).

Discussion

This chapter revealed four key findings. First, taxonomic and functional diversity metrics were lower upstream compared to downstream of the Hamilton Dam. Second, habitat characteristics, particularly flow regime and substrate availability, likely play an important role in structuring fish communities, although our habitat mapping techniques were inconclusive on determining the difference of substrate availability upstream vs. downstream. Third, we found limitations in comparing habitat mapping tools, with notable inconsistencies between sonars and sonar analysis tools. Finally, our results highlight the importance of comprehensive, site-specific assessments for understanding the ecological impacts of low-head dams in urban river systems.

When looking at taxonomic and functional diversity of the fish caught in our sampling area, these diversity metrics were lower upstream compared to downstream of the dam. This is likely for multiple reasons. First, with Hamilton Dam being the first barrier that fish encounter when traveling upstream in the Flint River this causes fish populations to pool downstream of the dam, although the habitat may not be the most ideal for them. Secondly, dams tend to create different environments upstream vs. downstream; a more lentic environment upstream and a more lotic environment downstream. This has also been found in conjunction with a decrease in habitat quality and altered fish and macroinvertebrate communities downstream of dams (Hastings et al., 2016c), as reflected by our fish metrics and macroinvertebrate scores (to be discussed in Chapter 2). Furthermore, the downstream location of the dam showed an overall negative relationship with functional diversity metrics, but in contrast did not show a significant effect on species richness or evenness. This implies that changes in the habitat due to the dam may not necessarily change the species of the area but will change the functional composition of species upstream vs. downstream of a dam. Smith et al., (2017) has found similar results in the Vermilion River, Danville, Illinois, with results indicating that low-head dams alter habitat and therefore fish functional assemblages. Water flow is found to have a significant impact on the physical habitat available to fish and macroinvertebrates. The addition of a velocity meter at the dam could help us understand if taxonomic diversity metrics would respond more to flow regime vs substrate availability (Poff & Allan, 1995).

Our taxonomic and diversity metrics decreased over time (from 2023 to 2024) when comparing both upstream and downstream. Our fish populations do naturally fluctuate, but this could have been due to the decreased amount of sampling days at sites directly surrounding the dam, from 2023 to 2024. The addition of 4 additional sites in 2024 due to the contamination

analysis did decrease the amount of sampling days directly at the dam from 23 days in 2023 to 14 days in 2024.

Locations upstream of dams tend to be more lentic while also being more homogenous in sediment composition (Alexandre & Almeida, 2010). Our habitat mapping was originally intended to provide information on if habitat availability was different from upstream vs downstream of the dam in terms of substrate hardness and variability. After visually analyzing the data from the sonars, it was unclear if there was a difference upstream vs. downstream of the dam. Additionally, PING-Mapper seemed to be biased toward soft and medium determinations, while BioBase seemed to be biased toward medium and hard determinations. In the future, substrate size should be sampled in the sonar mapped areas to gain a better understanding of the substrate availability and output of the sonar analysis systems. Further verification of the accuracy of these sonars, in conjunction with better ground truth hardness methods could help determine which of these sonars is more accurate or applicable for determining substrate availability of non-wadable rivers. Additionally, water flow is found to have a significant impact on the physical habitat available to fish and macroinvertebrates.

Our methods of habitat mapping could have also played a factor in the similarity of results within sonar outputs and perhaps could be re-evaluated. Sonars can be impacted by noise from a dam or from our gas motor. Additionally, the installation of the sonars was modified to be usable with our 12 ft-Jon boat. This required both sonars to be on the front, when typically, they are on the back of the boat and usually only one sonar is needed at a time. The sonars on the front of the boat could have caused water drag when moving down the river that may have been different than if they were on the back of the boat and could have impacted our results. The 2-3 sonar tracks per length of river did appear to provide more data for PING-Mapper, but did not

prove necessary for BioBase Maps, as BioBase Maps is programmed to analyze areas with missing data and fill in gaps accordingly. Lastly, the speed of the boat could have played a role in the quality of the data that was collected. Future directions could include a different set-up, operated by a quiet trolling motor on the front during data collection, and the sonars on the back of the boat.

BioBase Maps seemingly outperformed PING-Mapper at performing better than chance at modeling the ground truth. We found no evidence of agreement beyond chance between the two sonar classification methods. However, PING-Mapper may still be beneficial for scientific inquiries. PING-Mapper is a free open-source software, and the Humminbird unit used to collect the data is more cost effective and user friendly. In contrast, although BioBase Maps is more user friendly, the sonar is not as easily learned and more costly than the Humminbird unit. Perhaps in the future it would be more beneficial to compare sonar images over time for one sonar, vs comparing multiple sonars that are essentially not measuring the same thing. BioBase Maps provide hardness values on a unitless continuous hardness scale, where PING-Mapper provides distinct color-coded categories of bottom composition. Furthermore, it is important to understand if substrate size plays a role in the "hardness" values calculated by BioBase Maps.

This study provides comprehensive pre-restoration data on fish community composition and adds to the knowledge of instream habitat analysis methods surrounding a low-head dam in a highly urbanized river system. While taxonomic diversity showed no direct effects from the dam, functional diversity was different upstream suggesting that changes in flow and habitat from the dam affect ecological roles more than species identity. Our habitat mapping approach revealed challenges in comparing sonar derived metrics, emphasizing the need for consistent methodology and further research on physical habitat assessment methods. Overall, these results show that it is

important to consider functional traits, location, and the methods used when trying to understand how dams impact riverine ecosystems. As more dams are being removed, long-term comprehensive case studies such as this project are essential for making informed decisions that take into account the complexity and uniqueness of each riverine ecosystem.

To conclude, dams vary in size, function, age and structure. Rivers vary in their natural flow regime, substrate availability, location, land use and size. Due to the variance in dams and rivers, and the conflicting views on dam removals, each dam should be assessed in its own comprehensive way as all dams on rivers can impact the ecosystem in different ways. More long term and comprehensive studies on urban low-head dam removals can add to this growing need of research and can help develop a framework for low-head dam removals (Babbitt, 2002; Bellmore et al., 2017; Hart et al., 2002; Hastings et al., 2016a). A strong understanding of all aquatic species present, water velocity, substrate availability *and* substrate size, can help communities and managers make decisions about the best way to naturalize an area and use funding efficiently.

Table I-1. List of how determinations were derived for ground truthing the habitat mapped with multiple different sonars. Determinations were grouped into soft, medium and hard. A visual determination was made between "muck mud" (as soft), "muck sand" (as medium), and "boulder/cobble" (as hard) for the ground truth determinations and compared to determinations made by each sonar (HumminBird Helix7 G4 unit, Lowrance HDS-7 Live) and sonar analysis programs (PING-Mapper, BioBase Maps).

Type	**Ground Truthing**	**PING-Mapper**	**BioBase Maps**
Soft	"Muck Mud"	Fines Ripple (sand)	(0-.25) light tan
Medium	"Muck Sand"	Fines- Flat (gravel, and potentially sand, silt or mud)	(.25-.30) orange
Hard	Boulder/Cobble	Cobble Boulder, hard bottom (Bodine & Buscombe, 2023)	(.35-.5) dark red (BioBase Maps, 2024.)

Table I-2. Outcomes of model selection using the Akaike Information Criterion corrected for small sample sizes (AICc) of a mixed effects model evaluating predictors for location to the dam and sampling year. Model selection was based on AICc, and only models with $\Delta AIC < 4$ were averaged and presented in this table. Among the models evaluated, neither year nor location had a statistically significant effect on species richness or Shannon diversity index. Only location (upstream of the dam) had a statistically significant negative effect on functional dispersion as indicated by the P-value and 95% confidence intervals.

Explanatory variable	Coefficient	95% CI (lwr-upr)	P-value
	Species Richness		
Location Upstream	-5.0	-14.9 - 4.9	.324
Sample Year	- 0.75	-2.17 - 0.67	.302
	Shannon Diversity Index		
Location Upstream	0.01703	-2.92 - 2.95	0.991
Sample Year	-.09674	-0.431 – 0.238	0.571
	Functional Dispersion		
Location Upstream	-0.07100	-0.114 - -0.0305	0.00059

Table I-3. Weighted Cohen's Kappa results as a statistical measure of agreement between BioBase Maps and the ground truth data, PING-Mapper and the ground truth data, and between the two sonars, accounting for the degree of disagreement. A weighted matrix was used that gives more credit when disagreements are close (soft vs. medium) vs. determinations that are more different (soft vs. hard).

Type	Kappa Value	ASE	Z-value	P-value	95% CI
BioBase Maps and Ground Truth					
Unweighted	0.2632	0.1290	2.04	0.04137	0.010-0.516
Weighted	0.2727	0.1223	2.23	0.02575	0.033-0.512
PING-Mapper and Ground Truth					
Unweighted	0.08157	0.09502	0.8584	0.3907	-0.105-0.268
Weighted	0.02646	0.09616	0.2751	0.7832	-0.162-0.215
PING-Mapper and BioBase Maps					
Unweighted	-0.02703	0.10700	-0.2526	0.8006	-.237-0.183
Weighted	0.00000	0.08909	0.00000	1.0000	-0.175-0.175

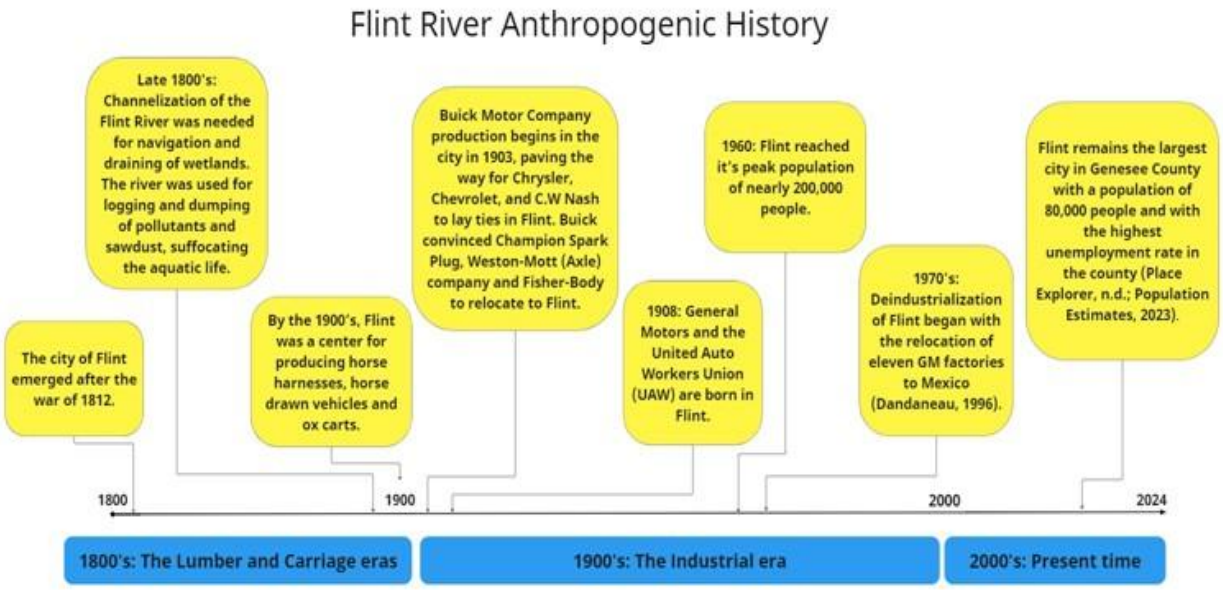


Figure 1. Timeline of anthropogenic influences on the Flint River and the City of Flint, Michigan, highlighting major industrial developments and population changes in the City of Flint. Key events include the channelization of the river in the late 1800s, the rise of the automobile industry in the early 1900s, peak urban population in 1960, and subsequent deindustrialization and demographic shifts. Information taken from (Dandaneau, 1996; Leonardi & Gruhn, 2001; U.S. Census Bureau, 2024).



Figure 2. Dams along the Flint River and its local tributaries. Nellis, Unsel, and Historical Crossroads Gristmill Dam are located on tributaries. The Hamilton Dam at the time of this study was a low-head dam consisting of a ladder type weir as the superstructure had been removed in 2018. The Hamilton Dam has no effective fish passage upstream and is the first barrier of the Flint River to potamodromous fishes. Utah Dam no longer impedes flow of fish movement. Mott Dam has no effective fish passage, while Holloway Dam fish passage is restricted to the sluice gates or downstream movement over the spillway. Fabri dam is not considered a dam that prevents fish passage or withholds large amounts of water and therefore was not included in the EGLE dam dataset used for this map. The water flows southwest from the Holloway Dam to the Hamilton Dam and begins flowing northwest, eventually flowing into the Shiawassee River, then the Saginaw River, and later into the Saginaw Bay in Lake Huron. Data from ArcGIS online data (Environment, Great Lakes, and Energy, 2023a) and basemap data from Esri 2012.

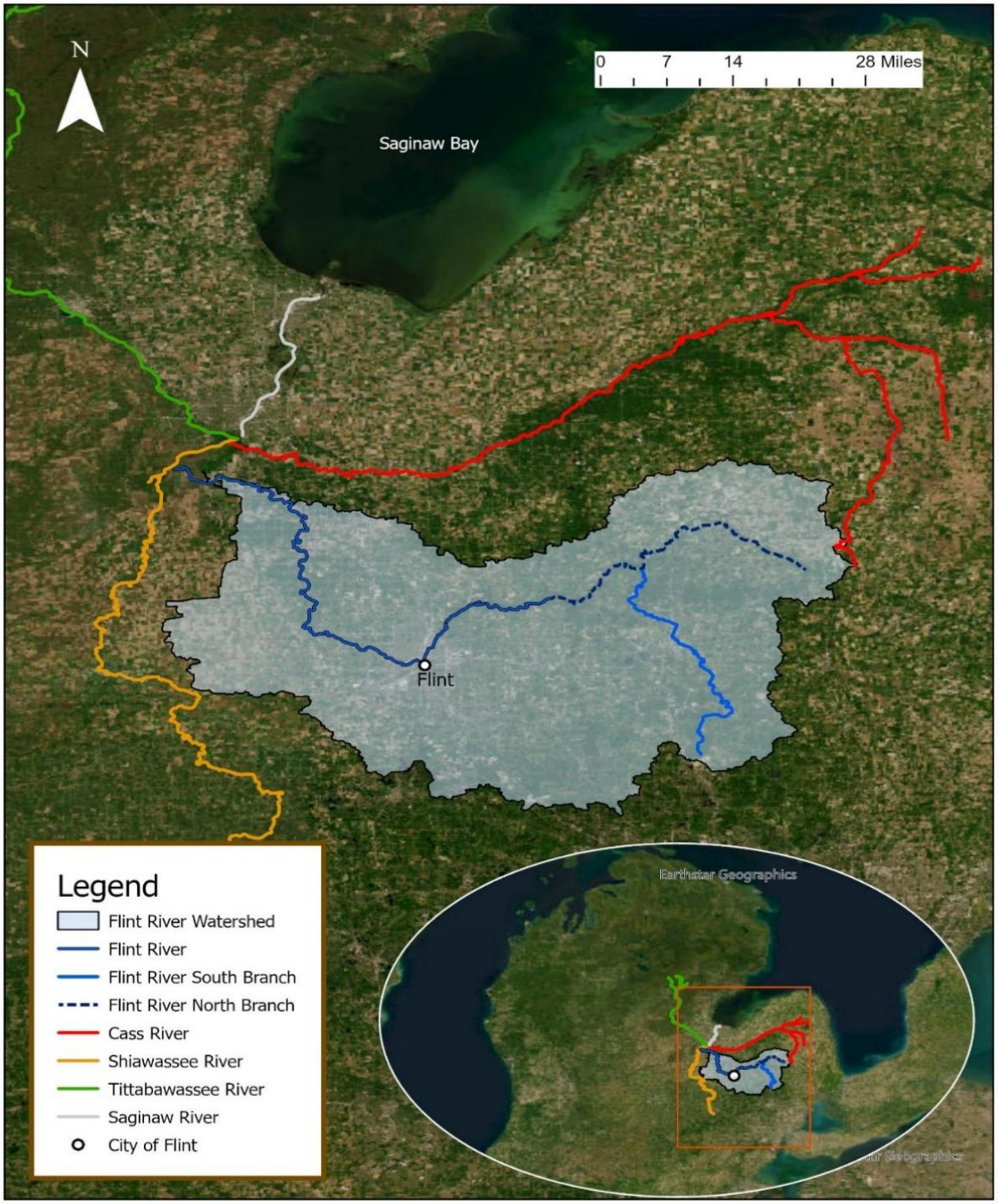


Figure I-1. Location of the Flint River and its watershed (gray), with the City of Flint indicated. The Flint (blue), Cass (red), Shiawassee (yellow), and Tittabawassee (green) Rivers merge to form the Saginaw River (white), which ultimately flows into the Saginaw Bay in Lake Huron. Map created in ArcGIS Pro (Esri, 2009).

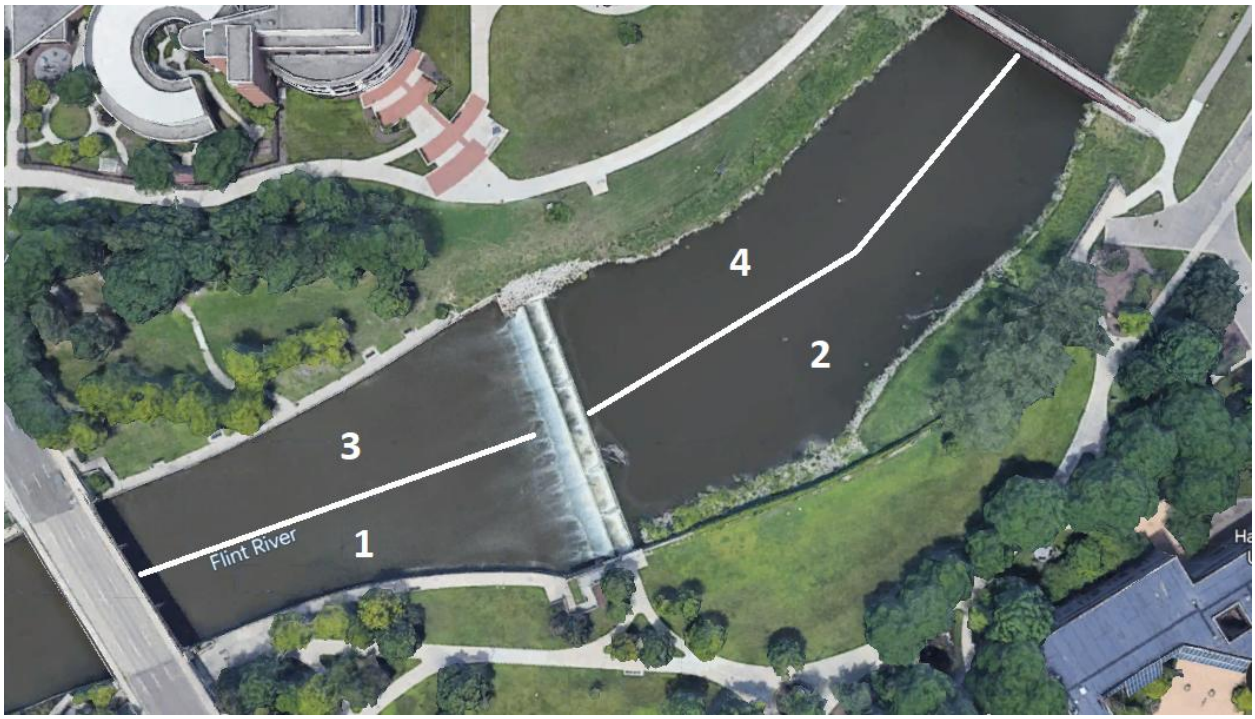


Figure I-1. Google Map aerial view of study sites on surrounding Hamilton Dam on University of Michigan-Flint campus, Flint Michigan. Sample sites 1-4 edited using the Paint application. Sites 1 and 3 are downstream of the Hamilton Dam. Sites 2 and 4 are upstream of the Hamilton Dam. Figure created by Chloe Summers and used with her permission.

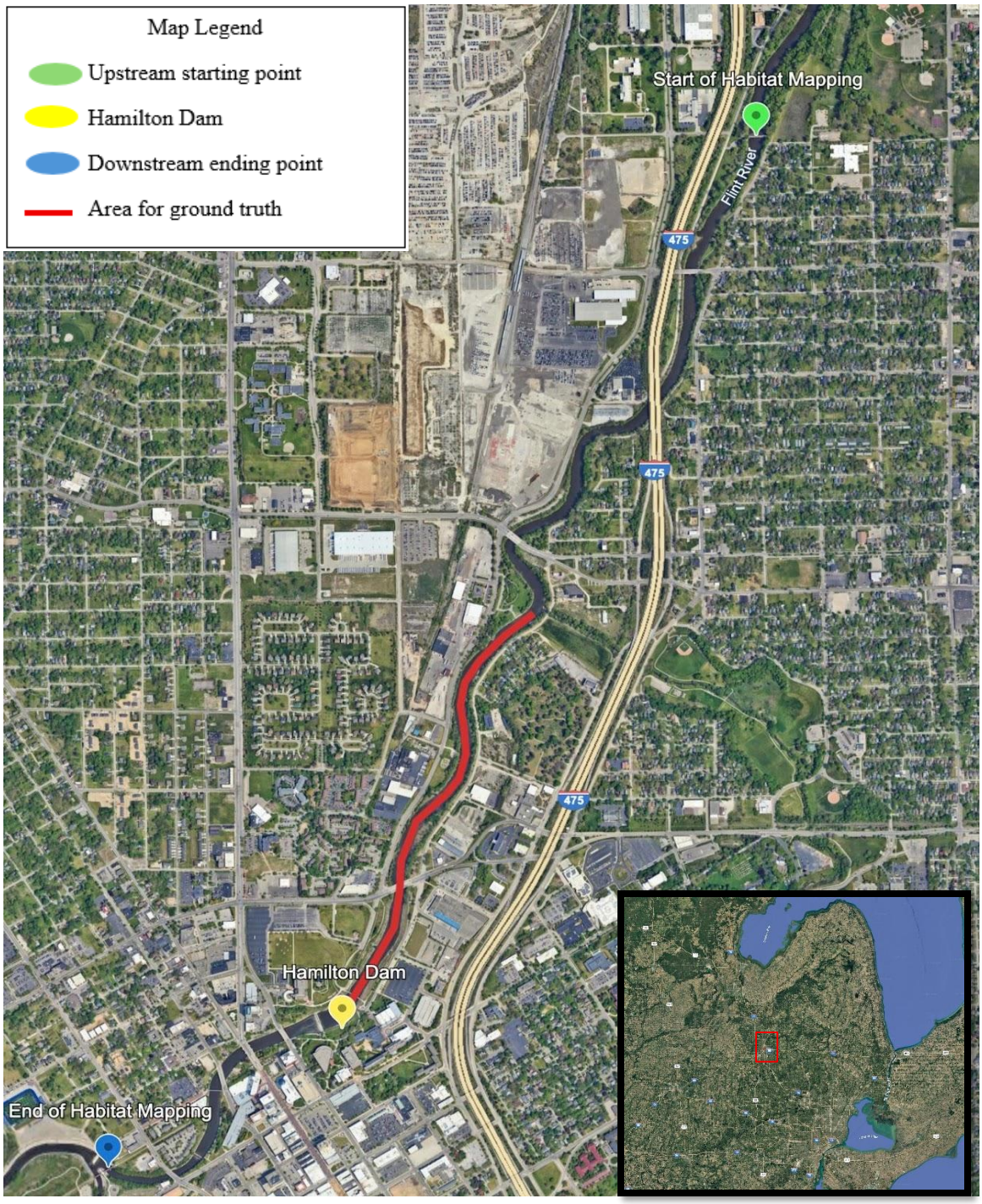


Figure I-2. Google Earth imagery of the location mapped for habitat mapping, and the location of ground truth sampling on the Flint River, Flint Michigan. The habitat mapping covered the entire restoration area, extending 2590 river meters upstream of Hamilton dam (green point), and 820 meters downstream of the dam (blue point). The Hamilton Dam is indicated in yellow. The ground truth sampling points are distributed along the red river segment, extending 1500 river meters upstream of the dam. Image created in Google Earth.



Figure I- 3. Raw data for fish caught in 2023 and 2024 upstream and downstream of Hamilton Dam, Flint Michigan. Species richness is the total number of species found in an area, where Shannon diversity index quantifies species diversity while considering both species richness and evenness of species abundances; with higher values indicating greater diversity. Functional dispersion is a measure of how functionally diverse fish species are in terms of ecological roles by measuring the degree of trait difference among species.

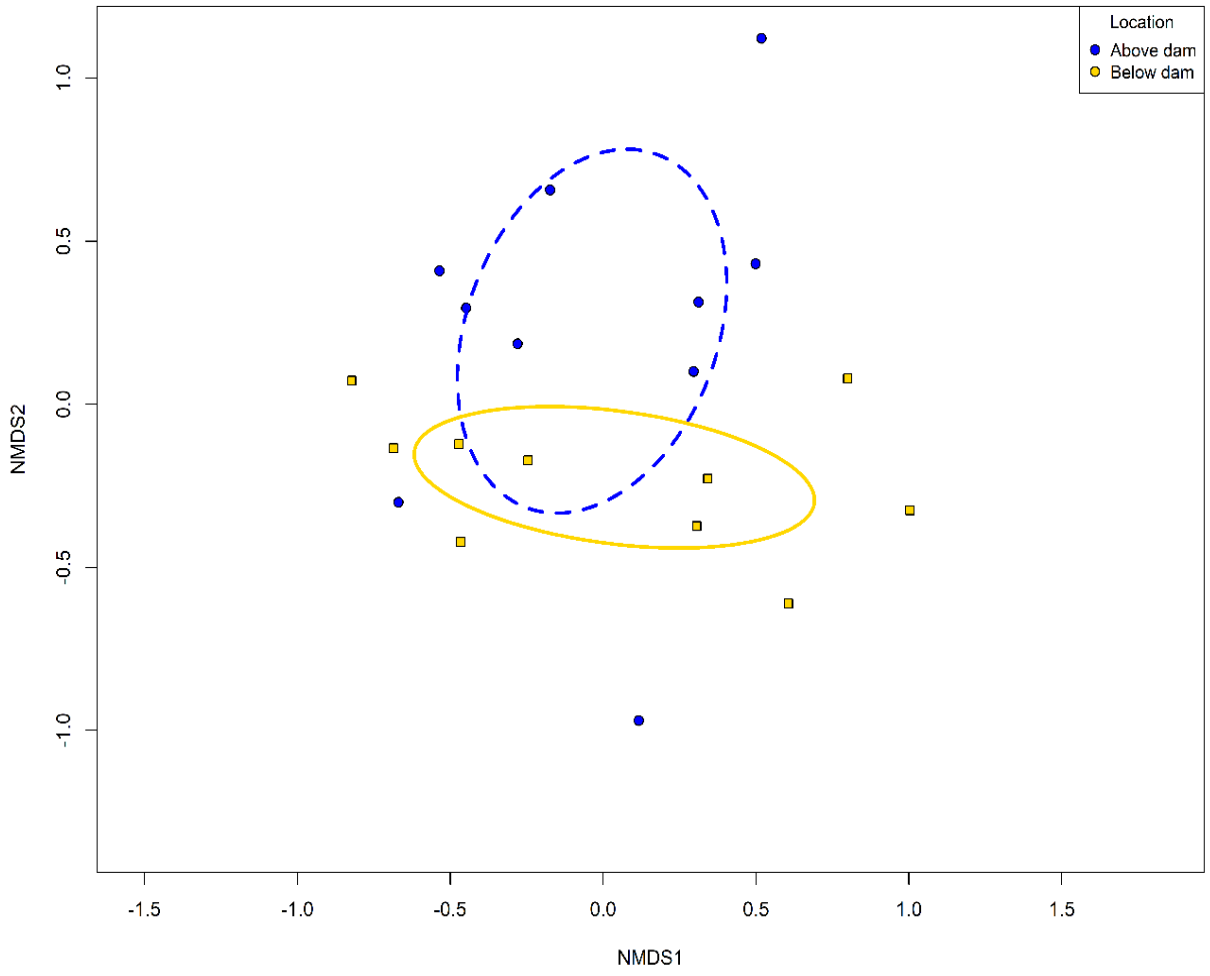


Figure I- 4. Visualization of differences in taxonomic diversity results between sites using Non-metric multidimensional scaling (NMDS) ordinations. The ellipses are ordination axes summarizing variation in trait composition. The overlapping ellipses indicate that taxonomic communities were not different above vs. below the dam for combined data from 2019, and 2021-2024. Permutational Multivariate Analysis of Variance (PERMANOVA) results = ($F = 0.658$, $R^2 = 0.19791$, $p = 0.919$).

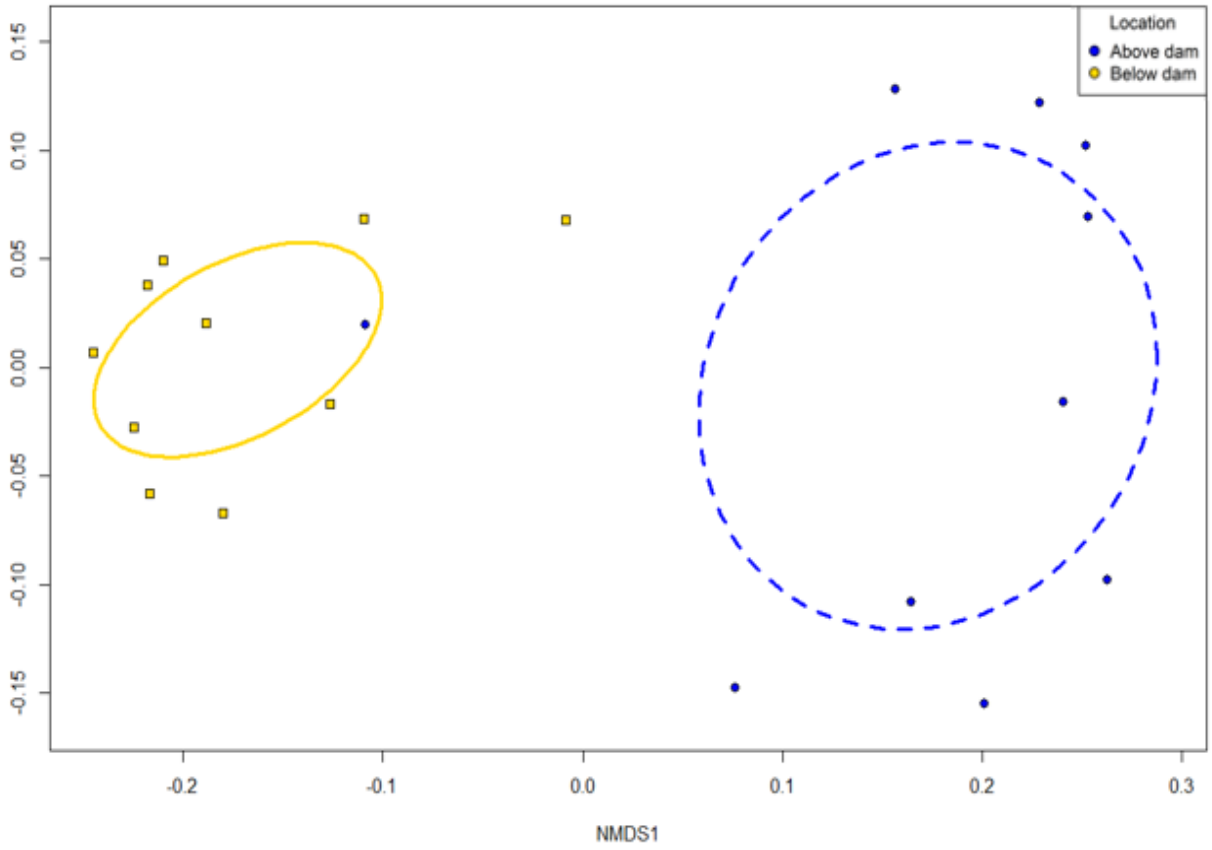


Figure I- 5. Visualization of differences in functional diversity results between sites using Non-metric multidimensional scaling (NMDS) ordinations. The ellipses are ordination axes summarizing variation in trait composition. The non-overlapping ellipses indicate that functional communities were different above vs. below the dam for combined data from 2019, and 2021-2024. Permutational Multivariate Analysis of Variance (PERMANOVA) results = ($F = 8.6235$, $R^2 = 0.7638$, $p = 0.002$).

II. Chapter 2

WATER QUALITY AND CONTAMINANT LEVELS IN WATER, RIVER SEDIMENT, AND FISH ALONG A GRADIENT OF ANTHROPOGENIC IMPACTS ON THE FLINT RIVER, MICHIGAN

Abstract

This study evaluated the effects of long-term urbanization and industrialization on the watershed of the Flint River, Michigan and its inhabitants. The objectives of this chapter were to; 1) determine if contaminant levels in fish, water and sediment vary in relation to media (water, sediment, fish) and location relative to toxic waste spills; and 2) assess how water quality as measured by benthic macroinvertebrates varies in relation to habitat assessments and contaminant levels in river sediment. To understand contaminant levels, we collected benthic sediment, surface water and fish tissue at 6 sites along a gradient of anthropogenic impacts along the Flint River, Michigan to be tested for heavy metals, pesticides, persistent organic pollutants and volatile organic compounds (VOCs). Of the 6 sites, 2 sites are directly above and below Hamilton Dam. Additional sites were selected downstream of Hamilton Dam, (an urban downstream, and non-urban downstream site) and similarly two sites were selected above Hamilton Dam (an urban upstream site and non-urban upstream site). In relation to the toxic waste spills that happened in 2022, 2023, and 2024 the Hamilton Dam upstream site is believed to be the most affected by this short-term pollution. We collected piscivores for heavy metal concerns, omnivores with the addition of rock bass (*Ambloplites rupestris*) for perfluorooctane sulfonic acid (PFOS) concerns, and benthivores were collected for DDT's and its metabolites (DDx). Additionally, we collected benthic macroinvertebrates at all sites as a form of water

quality indicators, in conjunction with completing habitat assessments to compare the health of the river as measured by both water quality indicators and habitat assessments. We investigated raw data of contaminant levels as well as studied the level of contaminants relative to other sites using Non-Parametric Multivariate Analysis of Variance (MANOVA). To interpret the contamination results, we integrated reference values for benthic sediment from MacDonald Sediment Quality Guideline (SQG), Canadian Sediment Quality Guidelines, Environment, Great Lakes, and Energy (EGLE) Water Resources Division Procedure 48, and rescinded U.S. EPA, Region 5 RCRA, Ecological Screening Levels as applicable. National Recommended Water Quality Criteria – Human Health Criteria Table, using the "Organism Only" category was used for surface water reference values. Lastly, for fish tissue we derived reference values from United States Environmental Protection Agency's (US EPA) Oral Reference Doses (RfD). Sediment results yielded that two sites suffered more in relation to other sites (1. Non-urban downstream and 4. Dam upstream), however only values for the Toxic Equivalency of PCBs, lead, cadmium and selenium yielded results above the provided reference value in sediment at the 4. Dam upstream site. In surface water, all recorded values for arsenic yielded results above the US EPA water criteria threshold. In piscivores, sites yielded results above RfD values for arsenic and mercury. No values of PFOS found in omnivores were above the RfD. For benthivore testing, 4,4-DDT was above the RfD at site 4. Dam upstream. In conclusion, the results from our contaminant data suggests that arsenic should be added to the contaminants monitored in the Michigan Eat Safe Fish Guides. More chemical contamination testing for water and sediment should take place to guide cleanup efforts for DDx, PCBs, arsenic, 1,4-dioxane, and vinyl chloride particularly at the 1. Non-urban downstream site, and 4. Dam upstream site as these sites had the most problematic sediment and water samples. Additionally, we found that

macroinvertebrate collection can be a quick and cost-effective way to understand the general health of an area. Our results indicate the effects of short-term and long-term anthropogenic effects from urbanization and industrialization on the Flint River Watershed and highlight the need for further testing to aid in restoration and cleanup efforts.

Introduction

The Flint River watershed has supported extensive industrial, municipal, and agricultural activities since the 19th century, contributing to legacy contamination and altered water quality in portions of the river (Leonardi & Gruhn, 2001; Wiitala & Krieger. R, 1963). Many factories in and around the City of Flint have been flagged for noncompliance or potential noncompliance with the United States Environmental Protection Agency (US EPA) regulations (United States Environmental Protection Agency, n.d.-d) and either relocated completely or were demolished (1990's-early 2000's). The last standing building of the most profitable facility in the history of General Motors, Chevy in the Hole, a 130-acre Flint River riverside factory complex (Hwang et al., 2013) was demolished in 2004, leaving contaminants behind that classified this area as a brownfield (Thelandbank.org, n.d.), and this factory is not unique in this manner (US EPA, n.d.-d). The site of Chevy in the Hole was sold to the City of Flint and is now a recreational park. The contamination and cement base remains, but the city has opted to cover the cement with soil and vegetation for the park. Much remediation was required to utilize this land; 1,500 trees were planted as a form of phytoremediation to sequester pollution in the soil (ECT, n.d.) and was considered part of the Flint River Restoration Project.

The Flint River received a poor reputation nationwide when a 2014 switch from Detroit River to the Flint River as a water source for the city ultimately resulted in lead, iron, and trihalomethane contamination of drinking water. However, it was not the fault of the Flint River. A temporary and cost-effective plan by the city backfired with the lack of proper water treatment and pipe corrosion control measures from the city (Dingle, 2016). The city has since switched back to using water from the Detroit River and has replaced over 97% of lead pipes within the city. Pipe replacements within the home were left to the resident's responsibility. As there is no safe level of lead to ingest, some residents still suffer from lack of clean drinking water, trust in

the municipal water system and bottled water is used on a regular basis. Although municipal water testing has tested within safe levels of heavy metals since July 2016 (Environment Great Lakes and Energy, 2023), many homes remain damaged from the improper water treatment, the pipe replacement that followed, and trust in the city has not been repaired.

Recent chemical spills in the Flint River include one by Lockhart Chemical that released roughly 20,000 gallons of petroleum-based toxic waste into the river in 2022, by ground water contamination of the city's storm drains (Edwards, 2022; Wimmer, 2025). There is evidence that the ecosystem has suffered at the hands of corporate negligence —in April 2019, before the Lockhart Chemical spill, there is evidence that both Flint sites of Lockhart Chemical were contaminating storm drains and Michigan Department of Environment, Great Lakes and Energy (EGLE) found elevated levels of perfluorooctane sulfonic acid (PFOS) (Kuberski, 2022). Cleanup efforts have been undertaken from federal and local responders; Lockhart Chemical has shut down the Flint facility and has now filed for bankruptcy (Edwards, 2022). Some cleanup efforts have occurred at the facility and in the river through the use of booms by response teams, but contaminants entering the sediments have seen no attempts to be removed. In June 2023, an unknown source allowed 10 gallons of petroleum-based substance to be leaked into the river via storm drains. This was followed by an additional three toxic waste spills in the same area as the previously mentioned spills in January, April and June of 2024 that remain of unknown source, amount and substance (Lorenz, 2024).

Due to groundwater concentrations tested after the Lockhart Chemical spill, we believe that 1,4-dioxane and vinyl chloride were signature chemicals in the 2022 Lockhart Chemical spill, along with arsenic and perfluorooctane sulfonic acid (PFOS). This testing provided concentrations for these contaminants above the Drinking Water or Ground Water to Surface

Water Interface (GSI) criteria directly after the spill. The contaminant 1,4-dioxane is a clear liquid that mixes easily with water, and a hazardous substance that humans can be exposed to in detergents, air, and water. This contaminant can enter the environment through manufacturing of chemicals and textiles, wastewater treatment plants and automotive coolant liquid ("1,4-dioxane," 2012; Ruhlman & Hill, 2016; Wilbur et al., 2012). The effects of 1,4-dioxane depend on the level of exposure and although this chemical will rapidly leave your body when in contact with it, it can also break down into other chemicals in the body. Small amounts that you may come into contact with in the environment and in consumer products would not have a lasting negative effect on humans (Wilbur et al., 2012). However, it has been found that repeated exposure to large amounts of 1,4-dioxane in animals can cause liver and kidney damage, and even cancer (Wilbur et al., 2012). The contaminant, vinyl chloride is a man-made, industrial chemical that is mainly used in plastic production. Humans can be exposed to vinyl chloride by vapor intrusion (vapors entering the air from contaminated soil or groundwater), and long-term exposure can lead to liver problems and increased risk of cancer in humans. In animals, it has been found to affect growth and development (Department of Health & Human Services, n.d.).

While the river has suffered from unintentional (but avoidable) chemical spills, there are 201 permitted waste discharge points throughout the watershed. These points consist of individual permits, wastewater discharge from petroleum or gasoline cleanup activities and other various wastewater discharges. Although permitted discharge must remain in compliance of water quality standards, non-point source pollution is not able to be regulated in this way and is a current water quality problem for the watershed. The biggest contributor to the degradation of water quality being water-aided erosion from agricultural land as stormwater and agriculture run off accelerates sediment and nutrient loading. We see other non-point source pollution in the

watershed from airborne pollution, fertilizers, pesticides, construction sites, road and railway crossings, golf courses, and irrigation.

A history of unregulated industrial, agricultural, regulated wastewater treatment plant, and human waste discharge, the unstable flow from urbanization of the river, and pollution during above watershed threshold flow periods all impact the water quality. Most of the land use in the Flint River watershed is either agricultural (43.0%) or developed (20.8%). Agriculture and urbanization have influenced the loss of natural vegetation, promoting erosion, and have altered the natural morphology of the river acting as sources of runoff and pollution (Fergus et al., 2023). Many pollutants require oxygen in the breakdown process, impacting the amount of dissolved oxygen available to aquatic life. Additionally, high-use agriculture areas will cause excess nutrient loading from runoff, which can cause an unnatural growth of aquatic vegetation causing further depletion of oxygen levels available to aquatic organisms (Leonardi & Gruhn, 2001). Due to contaminants found in waterbodies across the State of Michigan, the Michigan Department of Health and Human Services (MDHHS) monitors the edible portion of fish for pollutants. They use this information to provide guidance on which fish species are safe to eat from Michigan water bodies and publish them for each region in Eat Safe Fish Guides (MDHHS, 2023). The Michigan Eat Safe Fish Guidelines monitor long lasting, persistent, bioaccumulative and toxic chemicals: currently, mercury (Hg), Polychlorinated Biphenyls (PCBs), Dichloro-diphenyl-trichloroethane (DDT) and its derivatives (hereafter DDx), PFOS, selenium and toxaphene (MDHHS, 2023). Due to levels of mercury, PFOS, and PCBs in the Flint River, the Eat Safe Fish Guide states that citizens catching fish on the Flint River can consume one serving of rock bass (*Ambloplites rupestris*) a month, and six largemouth bass (*Micropterus salmoides)* and six smallmouth bass (*Micropterus dolomieu*) per year. Upstream of the Mott Dam, citizens

can consume one walleye (*Sander vitreus*) per month, four servings of black crappie *(Pomoxis nigromaculatus*), four servings of white crappie (*Pomoxis annularis*) and four servings of catfish (*Siluriformes spp.*) per month. Upstream, two servings of common carp (*Cyprinus carpio*) per month can be consumed but only one serving of common carp downstream per month (2023 Eat Safe Fish Guide for Southwest Michigan, 2023). It is important to note that servings should be spread out during the month, and not consumed in a single day (US EPA, 2000).

While historical industrial and agricultural activities throughout that watershed have led to a legacy of pollution, present day concerns focus on the persistent and bioaccumulative nature of these chemicals in aquatic food webs and human health. To understand ecological health effects on fish, macroinvertebrates, and therefore on humans, we studied heavy metals (mercury, lead, selenium, cadmium, arsenic), PFAS/PFOS, and persistent organic compounds (PCBs, DDx) in fish tissue, river water and benthic sediment. Aquatic organisms can encounter the previously mentioned contaminants through water, river sediment, and ingesting contaminated food sources. Contamination by fish with heavy metals and other contaminants can act as a vector for exposure to humans and other wildlife (Washington State Department of Health, n.d.). Some metals are naturally occurring in the human body and needed for bodily functions to some degree (selenium, copper and chromium) while other metals are non-essential in the human body and can be damaging at high concentrations (mercury, lead, cadmium; (Rahmani et al., 2018).

While heavy metals are naturally occurring in the environment, unnatural levels can be introduced by humans and bioaccumulate in the environment. Suryapratap & Vashishth (2024) states “A single gram of mercury which is a potent and widespread aquatic contaminant, can contaminate a 20-acre lake to the extent that fish from the lake may become unsafe for human consumption”. Toxic contaminants enter the waterways through agriculture, industrial and

municipal discharges, and storm water runoff. Bacteria and benthic macroinvertebrates encounter these contaminants, and when eaten, the fish encounter the contaminants. Humans may come into contact with these contaminants by consuming fish, touching contaminated surfaces, or inhaling airborne contamination. Once in contact with heavy metals, fish and humans can experience the accumulation of heavy metals in their organs and tissues, leading to health complications (Jaishankar et al., 2014).

Heavy metal pollution from industrial and sewage discharges poses widespread ecological and human health risks from the ability of metals to bioaccumulate and biomagnify in the environment (Baby et al., 2010). The heavy metal mercury is an important pollutant to test as it is an environmentally unpredictable, (Marziali et al., 2017) long-term, and persistent metal. The organic form of mercury found in the environment, methylmercury, can lead to neurological and cognitive damage in humans if ingested in unhealthy quantities (Carocci et al., 2014). Increased exposure to lead can lead to hair loss, cognitive disfunction and neurological, respiratory, cardiovascular and urinary diseases (Boskabady et al., 2018). Further, heavy metal (arsenic and cadmium) consumption in humans can lead to respiratory problems, cardiovascular diseases and renal dysfunction (Suryapratap & Vashishth, 2024). In addition to human health effects, arsenic can cause breathing and reproduction issues in fish (Ahmed et al., 2013; Taslima et al., 2022). Although selenium can be good for our health in moderation, too much exposure can lead to dizziness, gastrointestinal issues, and tremors (State of California OEHHA, 2022). Pregnant women, children and elderly are more susceptible to the adverse health effects that may be experienced when encountering an unhealthy amount of heavy metals.

Like heavy metals, organic chemicals also bioaccumulate in the food chain. The toxic and ecologically persistent chemicals, PCBs, enter the ecosystem globally from external

industrial sources (coolants, lubricants, plasticizers; McGoldrick & Murphy, 2016). As PCBs have been banned since 1979, the source is now believed to be the internal bioaccumulation in organisms and sediment, and atmospheric contamination (Carlson et al., 2010; Carlson & Swackhamer, 2006; MDHHS, 2023; Zhang et al., 2009). Dichloro-diphenyl-trichloroethane and its derivatives (DDx) are also persistent and bioaccumulate in the food chain, increasing in each trophic level (Siriwong et al., 2009). Humans can be exposed to DDx by eating meat, fish, dairy and even by breathing or touching contaminated products (Environmental Protection Agency, n.d.). High levels of exposure have led to vomiting, tremors and seizures (US EPA, 2025). The Center for Disease Control and prevention reports DDx as possible carcinogens that can be passed from mother through fetus and breast milk. Additionally, PFAS are found in waterbodies and are a family of persistent chemicals (PFOA and PFOS) that are used in consumer products and manufacturing worldwide (What is PFAS?, n.d.), and have been a known problem in the Flint River Watershed (**Figure II-1**). Humans mainly come into contact with PFAS by ingesting contaminated food or water (FlintRiver.org, n.d.). The persistent chemicals PCBs and DDx rest in the fat of fish, while mercury is stored in the muscle tissue. Thus, PCBs and DDx can be limited by trimming excess fat (Contaminants in Fish | Washington State Department of Health, n.d.). Following the Eat Safe Fish guidelines is crucial for the health of citizens, as these contaminants cannot be cooked out of food entirely (PFAS in the Flint River Watershed, n.d.).

An important addition to a comprehensive ecological health analysis is the collection and analysis of macroinvertebrates as a water quality and habitat health indicator. Macroinvertebrate species have varying levels of sensitivity to water quality (Hashim et al., 2023) and thus are used as indicator organisms. Chemical and heavy metal pollution can affect the macroinvertebrate species assemblages of an area, as some species simply cannot survive in polluted areas

(Blachuta et al., 2014; Fergus et al., 2023; Marziali et al., 2017; Qu et al., 2010). Additionally, substrate size heterogeneity and availability will play a role in the macroinvertebrates present in an area (Poff & Hart, 2002) as well as habitat contributions like presence of riffles and pools (Marziali et al., 2017). The Flint River Watershed Coalition (FRWC), EGLE and the Flint River Ecology study have been collecting macroinvertebrates in spring and fall on the Flint River at some sites since as early as 1999. Although there has been an increase in macroinvertebrate scores over the years, there still remains lower macroinvertebrate scores in the channelized downstream reach of the Hamilton Dam in comparison to the more naturalized upstream reach (C. Summers, University of Michigan-Flint, unpublished data; *Benthic Monitoring, n.d.; Qualitative Biological and Habitat Survey Protocols for Wadeable Streams and Rivers, 2024)*. Macroinvertebrates serve as both water quality bioindicators and as a key food source for fish, thus understanding their response to contamination provides important insights into broader ecosystem impacts.

This chapter aims to understand the current and historical anthropogenic impacts on fish and macroinvertebrate communities. The objectives of this chapter are to; 1) determine if contaminant levels in fish, water and sediment vary in relation to media (water, sediment, fish) and location relative to toxic waste spills; and 2) assess how water quality as measured by benthic macroinvertebrates varies in relation to habitat assessments and contaminant levels in river sediment.

Methods

Study Area

The study area for Chapter 2 encompasses six sites along the Flint River in Genesee County, Michigan, centered around the two primary sites located immediately upstream and

downstream of the Hamilton Dam, as seen in Chapter 1. To represent a gradient of anthropogenic impacts across urban and non-urban settings along the Flint River, four additional reference sites were included: two upstream and two downstream of Hamilton Dam (**Figure II-2**). Downstream reference sites included an urban site located at Mott Park in the City of Flint and a non-urban site at Flushing Township Nature Park, the farthest downstream location in the study. Upstream reference sites included an urban site at Stepping Stone Falls (~700 river meters downstream of Mott Dam), a public park in Flint, and a non-urban site at Irish Road Fishing Site in Davison, the farthest upstream location (**Figure II-2**).

Contaminants

For contaminant analysis samples, we collected fish tissue, surface water and benthic river sediment from May-August 2024. Two replicates of river water and benthic sediment samples were collected from each site (12 total/media). We aimed to collect 6 piscivores, 6 benthivores, and 6 panfish at each site. Fish were obtained and processed by methods outlined in Chapter 1 (with no external or internal tagging needed) and then euthanized with an overdose of tricaine methanesulfonate and filleted to obtain tissue samples. Additionally, 2 fish of each feeding group were added to this testing that were collected prior to the toxic waste spills at the 3 and 4 dam upstream/dam downstream sites and preserved at -18 ℃. Fish tissue samples were wrapped in tin foil, labeled, and frozen at -18 ℃ until ready to be shipped for external chemical analysis. We followed MDHHS procedures when collecting, filleting, and storing fish to ensure our data could be comparable with the Eat Safe Fish data produced by MDHHS.

Surface water samples and benthic sediment samples were collected on June 21st, 2024. Water and sediment sample bottles were provided by the external testing facility: Pace Analytical Labs, Green Bay, WI. Surface water was collected by wading out to the middle of the

river and collecting surface water directly into the provided sample bottles. Benthic sediment samples were collected by obtaining surficial benthic sediment by shovel scoop and adding sediment to the provided sample bottles, being cautious to omit large debris as instructed.

Fish tissue was collected from May-August until catch goals were met or exceeded. We tested fish tissue for heavy metals (mercury, cadmium, lead, selenium, arsenic), PCB (Dioxin-like congeners), DDx, and PFOS. Surface water and benthic sediment samples were tested for the aforementioned chemicals with the addition of 1,4-dioxane and vinyl chloride as they were chemicals found after the 2022 Lockhart Chemical spill; however these contaminants cannot be readily tested in fish tissue. To test mercury (Hg) and other heavy metals, a top predator fish (piscivore) was targeted as this is a bioaccumulative chemical. Piscivorous fish diet consists of mostly other fish (bass, walleye). To test for PCBs and DDx, a bottom feeding fish (benthivore) was targeted (carp, sucker or catfish) as these chemicals tend to settle into sediments (Washington State Department of Health, n.d.). To test for PFOS, panfish (omnivore) with the addition of rock bass were targeted as PFOS tends to reside in the lower trophic levels of rivers (Fish Tissue PFAS Sampling , 2019), Omnivorous fish feed on both plants and animals, and testing omnivores allowed us to stay consistent with Michigan Eat Safe Fish Guides (Michigan Department of Environmental Quality, 2019; MDHHS, 2023).

For interpreting raw data values, reference values for 4,4'-DDT, mercury, arsenic, cadmium, and lead were derived for benthic sediment from MacDonald Sediment Quality Guideline (SQG), specifically the consensus-based Probable Effects Concentrations (PEC) (Macdonald et al., 2000). For the Toxic Equivalency of PCBs, (TEQPCB; a weighted toxicity based on overall makeup of PCBs in sample), we derived reference values for benthic sediment from the 2,3,7,8-TCDD pgTEQ/g (ppt) referenced in the Canadian Sediment Quality Guidelines,

2002. We derived benthic sediment reference values for selenium were referenced from the EGLE Water Resources Division Procedure 48. We derived reference values for vinyl chloride and 1,4-dioxane from guidelines of rescinded U.S. EPA, Region 5 RCRA, Ecological Screening Levels (U.S. EPA, 2003). There are no direct sediment guidelines for vinyl chloride or 1,4-dioxane (vinyl chloride typically does not accumulate in sediment due to its high volatility), so the rescinded US EPA values were used as reference and are derived by using an equilibrium partitioning (EqP) equation and the corresponding Ecological Screening Level. As PFOS does not have sediment quality guidelines, no reference comparison can be made at this time. Reference threshold values for surface water were obtained from the National Recommended Water Quality Criteria – Human Health Criteria Table, using the "Organism Only" category which indicates the health of the water assuming that people are not drinking the water, but still consuming fish from it over their lifetime (EPA, 2024; US EPA, n.d.-a). We derived reference values for fish tissue from Oral Reference Doses (RfD), specifically for mammalian exposure, but plant and avian exposure is also calculated for nearly all contaminants tested and could be lower or higher than the mammalian value depending on the contaminant (US EPA, n.d.-b).

Macroinvertebrates

At all six sites, we collected macroinvertebrates with a flat top "D" Lamotte net using methods and scored the macroinvertebrates using the FRWC protocol with modifications to adapt to non-wadable rivers. Spring macroinvertebrate collection occurred in May of 2023 and 2024 to stay consistent with previous sampling from this project and FRWC. Where possible, shallower areas along the riverbanks were sampled for macroinvertebrates, although the river itself may not have been completely wadable. For completely non-wadable sites such as the 3. Downstream Dam site, our modified method consisted of using a 12-foot Jon boat with

extendable “D” Lamotte nets employed over the edge of the boat to in areas where the nets could reach the benthic sediment. Habitat assessments were completed at each of the 6 sites following the Michigan Department of Environmental Quality Procedure 51 for wadable and non-wadable streams as applicable (EGLE, 2024). This survey assesses substrate and instream vegetation cover, channel morphology, riparian bank structure, and riffle/run stream presence on a 100-point scale with scores indicating excellent (100-84), good (83-56), marginal (55-28) and poor (27-0). These thresholds within the habitat assessments are utilized in EGLE's survey reports to categorize habitat quality specifically in Michigan's streams and rivers.

Analyses of Contaminants

All analysis was completed in R version 4.4.3 (R Development Core Team 2025). The Toxic Equivalency of PCBs was used in analysis. There was no analysis of TEQs from dioxins/furans (TEQD/F) completed. If values were higher for one site, this does not necessarily mean that it had more PCBs but could indicate that it has presence of more harmful PCBs. Values below the Method Detection Limit (MDL) were considered to be zero. Below the MDL does not mean zero, but so low that the instrument used to test cannot accurately detect the concentration due to the standards and methods developed for that instrument. To test the relative effects of contaminants by site, we used Non-Parametric Multivariate Analysis of Variance (MANOVA) using the package npmv. The contaminants tested were the multiple independent variables, and the dependent variable being location of the site. To avoid multicollinearity, we removed some contaminants from the models (lead, cadmium, selenium, DDx, PFAS) with variance inflation factors (VIF) > 6. To quantify the magnitude of effects across sites, we computed nonparametric relative effects and showed these values on line graphs with help from ggplot2 *(*Wickham, 2016) and ggpubr (Kassambara, 2025). We completed

statistical tests to determine if values returned for each media and each contaminant were above the provided threshold values. For benthic sediment, we calculated Probable Effects Concentration quotients (PECq) as outlined by MacDonald (2000). As sediment benchmarks are not available in MacDonald (2000) for TEQPCB, PFOS, 1,4-dioxane, and vinyl chloride, we instead applied one-sided exact binomial tests. To evaluate surface water, we conducted one-sided exact binomial tests to assess whether the proportion of contaminant screening value exceedances was greater than the expected background exceedance probability ($p_0 = 0.10$). Tests were performed separately for each sampling site, with the null hypothesis that exceedance rates did not differ from 10%, consistent with Section 303(d) of the Clean Water Act (Smith et al., 2001). The same one-sided exact binomial test was also applied to fish tissue analysis, as no standardized statistical approaches currently exist for evaluating contaminant exceedances across multiple species and contaminants.

Analyses of Macroinvertebrates

To understand if habitat assessment scores or contaminant levels found in fish, water, and sediment predict macroinvertebrate scores, we used the dredge function from the MuMIn package to perform model selection via Akiake Information Criterion corrected for small sample sizes (AICc) (Bartoń, 2025). We used the global model: Macroinvertebrate scores ~ Site type (urban or non-urban) + contaminant levels + habitat scores. To avoid multicollinearity, we removed variables with variance inflation factors (VIF) > 6.

Results

Contaminants

While contaminants were detected across all media tested (river water, benthic sediment and fish tissue), the interpretation of these results require comparison against established

reference values to understand if the presence of these contaminants are at ecologically unsafe levels. For this reason, relative effects graphs are shown merely to provide comparison points between the sites. Raw data from the 2024 collection is shown in the form of tables, with the applicable reference values provided for further understanding of whether our contaminant data is of concern. Statistical tests were completed to determine if replicates at each site for each media were statistically above the threshold value of concern. To assist in visualizing spatial trends in the contaminant analysis along the Flint River, a schematic was developed to summarize the key findings across all study sites presented with the direction of flow of the Flint River (**Figure II-4**).

Sediment

To address multicollinearity in the statistical analysis of river sediment data, some variables had to be removed from the relative effects model (DDx, 1,4-dioxane, and vinyl chloride) but are shown in the raw data tables. The final model included arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), selenium (Se), TEQPCB, and PFOS—all of which yielded significant differences both across contaminants and between sampling sites ($P < 0.05$).

In relation to other sites, values of heavy metals (arsenic, mercury, selenium, lead and cadmium) were statistically higher at the 4. Dam upstream site in benthic sediment (**Figure II-5**). Of all the contaminants, the highest concentrations in sediment (except for PFOS, DDx, 1,4-dioxane and vinyl chloride) were found at the 4. Dam upstream site (**Table II-3, Table II-4**). Of the raw data values in benthic sediment, the 4. Dam upstream site indicated the highest values of mercury (0.20-0.22 ppb), arsenic (24.6-25.0 ppb), cadmium (5.90-7.85 ppb), lead (186-533 ppb) and selenium (1.7-2.1 ppb). However, only values for Pb, Cd, Se, and TEQPCB at this site were statistically significant above the provided reference values. Concentrations for lead at site 4.

Dam upstream yielded elevated values for both replicates tested compared to the reference value (concentrations: 186 ppm, 553 ppm; reference value: 128 ppm). Site 4. Dam upstream produced two concentrations above the PEC for cadmium (4.98 ppb). TEQPCB values at the 4. Dam upstream site were double the provided reference values (concentrations: 55.4 ppt, 115 ppt, reference value: 21.5 ppt), with no other sites reaching over 2 ppt.

The 1. Non-urban downstream site produced the highest levels of 1,4-dioxane (104-116 ppb), and vinyl chloride (33.0-38.2 ppb). Sites 1. Non-urban downstream and 2. Urban downstream sites produced the highest values for PFOS, however no reference values are currently available for PFOS in sediment (**Table II-3**). Across all sites, no results for 1,4-dioxane, vinyl chloride, DDx or mercury were observed above the provided reference values. However, the 4. Dam upstream site yielded concentrations of selenium nearing the provided reference value (concentration: 2.1 ppb; reference value: 2.9 ppb).

Surface Water

Values for cadmium, selenium, mercury, DDx, vinyl chloride and 1,4-dioxane levels were below the external lab's Method Detection Limit (MDL), and thus have been recorded as zero, therefore omitted from the raw dataset (Table II-5). A value below the MDL does not indicate zero but indicates a value so low that the instrument cannot accurately determine the concentration. The external lab's instrument MDL's are all below the threshold values for these contaminants found in surface water as provided by the EPA's National Recommended Water Quality Criteria for human health, except for the suite of DDx tested. The MDL for DDx ranges from 0.064-0.087 ppb, while the reference values for the suite of DDx range from 0.000018-0.00012 ppb.

Variables shown in the relative effects graphs were significantly different between each other and between sites (P = 0.003). The 4. Dam upstream site shows the largest relative effect of arsenic and TEQPCB (**Table II-5**) relative to other sites. While no results for TEQPCBs were above the reference value across sites, all arsenic values returned in surface water at all sites were above the EPA water criteria threshold (0.14 ppb; **Figure II-6)**. Results for PFOS in surface water proved statistically significant over the reference value at the 2. Urban downstream site only. Although there are no current EPA reference values for lead to compare results to, lead was only found in 3 of 12 water samples, 1 of 2 replicates at the 2. Urban downstream site and both replicates at the 6. Non-urban upstream site (ranging from 1.1-1.2 ppb).

Piscivores

A total of 36 piscivores were tested for heavy metals. The urban upstream site yielded the lowest mean levels for heavy metals tested in fish (**Figure II-7**). Adjustments were made to remove contaminants with high multicollinearity, values with VIF > 6. The remaining variables in the model (mercury, arsenic, cadmium, and selenium) were significantly different between each other and between sites (P < .05). Of all fish tested at all sites, lead was not found in any piscivores, and cadmium was found in only one of the 36 piscivore samples (a 3. Dam downstream sample = 0.22 ppb). All fish produced levels of selenium (range: 0.16-0.39 ppb) but were not over the RfD of 5 ppb. While all piscivore samples contained arsenic, arsenic was highest at the 4. Dam upstream site, yet all samples that returned arsenic values above the MDL were over the RfD (24 of 36 total samples). Of the 36 samples that were tested for Hg, 20 of 36 samples were over the RfD with 5 of 6 piscivores at the 6. Non-urban upstream site and 3. Dam downstream sites reading over the RfD (**Table II-6**).

Omnivores

A total of 38 omnivores were tested for PFOS. Statistical analysis reveals differences between the sites in contaminant levels ($p < 0.001$). Of the omnivores tested PFOS in 2024, PFOS was found in the highest concentrations in the urban downstream site ranging from 0.557-25.8 pg/L. Although PFOS was present in all samples tested (overall range of 0.0186-25.8 pg/L), no values were above the EPA Oral Reference Dose of 1000 pg/L (**Table II-7**). At the 6. Non-urban upstream site, PFOS was highest relative to other sites (**Figure II-8)**.

Benthivores

Of the 38 total benthivores tested for DDx and PCBs in 2024, the 2. Urban downstream site had highest TEQPCB (0.557-25.8 pg/L) followed by the 3. Dam downstream site (0.0186-9.51 pg/L). Of the 3 benthivores that were collected prior to the toxic waste spills, TEQPCB produced results higher than testing completed relative to benthivores collected at all sites in 2024 (0.775-15.4 pg/L) Of the DDx tested, 4,4'-DDE was the derivative that was most found: in 21 of 36 total piscivore samples ranging from (5.6-12 ppb) and was found at all 6 sites (**Table II-8**; **Figure II-9)**. Due to the large suite of DDx tested, only 2,4- DDE, 4,4-DDE 4,4-DDD, and 4,4-DDT were reported as raw values, as they were found the most in benthivore samples. Statistical analysis reveals no differences between the sites in contaminant levels ($P > 0.05$).

Macroinvertebrates and Habitat Scores

Although we did not statistically test these results, the results of macroinvertebrate collections indicate that scores have increased downstream of the dam (2023 Spring Score= 2.1 (poor), 2024 Spring Score= 9.3 (poor)), while scores upstream of the dam have remained similar (2023 Spring Score= 21.5 (fair), 2024 Spring Score= 21.4 (fair)) throughout this study. Scores upstream of the dam have historically been higher than downstream of the dam. The upstream

and downstream non-urban reference points, as well as the urban downstream site indicated a "good" habitat assessment score, where the sites surrounding the dam are categorized as "poor" (**Table II-1**). This indicates that our sites positively reflect a gradient of anthropogenic disturbances on the river, and the presence of the urbanization and the Hamilton Dam impact macroinvertebrate species presence.

There is a clear indication that the quality of the habitat as outlined in the habitat assessments does play a significant role in the species of macroinvertebrates that can live in the area (**Figure II-3**). Of the 30 models evaluated using AICc-based model selection, two top models emerged as the only candidates with ΔAICc values < 4 and VIF < 6. Only models with ΔAICc < 4 were averaged. The best-supported model was: MacroScoreFRWC ~ Arsenic + TEQPCB + Mercury + Habitat Assessment Score (AICc = 87.9, ΔAICc = 0, K = 3, log-likelihood = -39.425, ω = 0.828). The second-ranked model included Site Type as an additional predictor: MacroScoreFRWC ~ Arsenic + TEQPCB + Mercury + Habitat Assessment Score + Site Type (AICc = 91.0, ΔAICc = 3.4, K = 4, log-likelihood = -38.638, ω = 0.172; **Table II-2**). Across both models, habitat assessment score was the only variable with a significant effect (positive) on the macroinvertebrate score, as indicated by its 95% confidence interval (2.960—21.035). The confidence intervals for site type ranged from -9.475—28.175.

Discussion

This chapter revealed four key findings that can aid in cleanup efforts in relation to the restoration event on the Flint River and can be applied to any low-head dam in an urban temperate riverine region. First, benthic sediment testing revealed that sites 1. Non-urban downstream and 4. Dam upstream suffered most relative to other sites tested. However, only

cadmium, lead, selenium and PCBs yielded raw values statistically above the threshold reference values at site 4. Dam upstream. Second, in surface water samples we found values of arsenic over the provided reference values. Third, we did not find any results that imply that Flint water crisis had a significant effect on the Flint River ecosystem, but it appears that the 2022 Lockhart Chemical spill did. Lastly, we found that macroinvertebrate scoring correlates with the health of the aquatic habitat as tested by habitat assessments.

Benthic sediment contamination results indicate that the 4. Dam upstream and 1. Non-urban downstream sites suffer the most relative to other sites. The 4. Dam upstream site had the highest levels for heavy metals (lead, selenium, cadmium, mercury and arsenic), and TEQPCBs. However, only raw values for cadmium, lead selenium, and TEQPCBs, were above the reference values at this site. Further investigation and testing are needed for lead, cadmium, and PCBs, at the 4. Dam upstream site to aid in restoration and cleanup efforts. The 1. Non-urban downstream site provided the highest values of 4,4'- and 2,4'-DDT, 1,4-dioxane, and vinyl chloride relative to other sites. No concentrations reached above the reference value, although concentrations for 1,4-dioxane reached very near to the reference value. We added vinyl chloride and 1,4-dioxane to the matrix of contaminants tested as they are believed to be signature chemicals in the 2022 Lockhart Chemical spill and did test above GSI values directly after the spill. The presence of these chemicals imply that these chemicals could have been released in the 20,000 gallons of toxic waste that was spilled into the river by this companies' degraded infrastructure and have been carried downstream. On the other hand, there are potentially other sources of contamination within the area of this site that could have played a role (municipal, industrial, and stormwater drains), but this would be difficult to determine without significantly more testing at additional sites over time. Additionally, this could also be indicative that 4,4' and 2,4' DDT, 1,4-dioxane,

and vinyl chloride all have a potential contamination source somewhere along the Flint River between our 4. Dam upstream site and 1. Non-urban downstream site. Further benthic sediment testing is needed over time to determine if there is a potential source for these chemicals outside of the 2022 toxic waste spill, or if remnants of this spill remain in the river.

As 1,4-dioxane and vinyl chloride are not regularly tested for ecological purposes, there are no reference values available for sediment, only rescinded US EPA Ecological Screening Levels (ESL). The Agency for Toxic Substances and Disease Registry states that 1,4-dioxane is a clear liquid that mixes well with water and will not reside in soil, and the North Carolina Water Resources Division states that 1,4-dioxane is tested in surface water ("1,4-Dioxane" 2012; Ruhlman & Hill, 2016). This calls for further research, as we did not find presence of 1,4-dioxane in surface water, however we did find it in benthic sediment. Although the discovered values did not reach over the rescinded U.S EPA ESL, these contaminants are not naturally occurring and should still spark discussion on cleanup efforts within the Flint River.

Of the water testing completed, we found that values of PFOS, arsenic and TEQPCBs were above the thresholds provided in **Table II-5**, with 12 of 12 water samples collected showing above the 0.14 ppb water criteria threshold for arsenic. The detection of arsenic in all water samples indicates that this is a persistent problem throughout the Flint River and not localized to one site or land use. Arsenic is naturally occurring in the environment in rocks and sediment but can also be introduced in unnatural amounts by various industries and pesticide use (Ayotte et al., 2017). Michigan is known for having naturally high arsenic levels in ground water (United States Geological Survey, 2000), and the National Recommended Water Quality Criteria for arsenic states that a value above 0.14 ppb is hazardous to humans consuming fish from this water over their lifetime. The US EPA states that the maximum contaminant level for arsenic in

drinking water assuming long term exposure is 10 ppb. Our values range from 4.7-5.6 ppb, which may indicate that the river water is safe to come into contact with, but due to the bioaccumulative nature of arsenic, fish consumption should be monitored for arsenic in the Flint River. This would aid in the public knowledge of residents in the area in understanding the potential harm of consuming fish from the river regularly. Of all piscivores that were tested for arsenic, if there was presence of arsenic found above the external labs MDL, all values found were above the oral reference dose of 0.06 ppb (our discovered range is 0.1-0.38 ppb). In piscivores, mercury results were above the 0.10 ppb oral reference dose in 20 of 36 samples, however mercury is already a monitored contaminant for the Michigan Eat Safe Fish guides (MDHHS, 2023).

For fish collected in 2024, the 4. Dam upstream site shows some of the highest values and concerns, which coincides with our expectations due to urbanization and chemical spills. This is reflective of contaminants moving to the 3. Downstream dam location. However, our most non-urban downstream site (1. Non-urban downstream) was still affected by DDx, 1,4-dioxane, and vinyl chloride and the most upstream non-urban site (6. Non-urban upstream) suffers from the highest values of mercury in piscivores. This could be indicative that these contaminants traveled downstream over time (**Figure II-4**), or there is another source for them in the non-urban areas.

Although Flint has suffered from a drinking water crisis involving the contamination of lead, our data suggests that an increased amount of lead in drinking water over many years is not reflected in the lead levels found in the aquatic environment within the City of Flint. This helps support the fact that lead in drinking water in Flint during the water crisis was due to poor corrosion control methods used when the Flint River was used as the drinking water source that

caused lead from pipes to leach into the drinking water, and is not the fault of the river itself. The only lead found across water, sediment or fish tissue appeared in surface water at 1 of 2 replicates at the 2. Urban downstream site, and both replicates at the 6. Non-urban upstream site. Although drinking water contamination can lead to increased contamination in the environment, studies state that exposure to humans and wildlife is more common from direct contact with contaminated soil or airborne dust in the dryer months of the year (Levin et al., 2020; McLean, 2016).

You may notice that the 5. Urban upstream site does not hold any determinations for the highest contaminants across sediment, water, or fish tissue (**Figure II-4**). As this was an urban reference point that is ~700 river meters downstream of Mott Dam, this is particularly interesting. This could indicate that the presence of a dam does not necessarily impact the number of contaminants found in an area and contamination is likely due to other sources around the dam, such as land use.

Macroinvertebrates have been commonly collected as a form of water quality indicators and to understand overall habitat quality in streams and rivers (Mcintosh, 2007; Reynoldson et al., 1997). We find support for this in our data with macroinvertebrate scores showing a linear correlation with habitat assessment scores (**Figure II-3, Table II-2**). In conclusion, if a cost-effective ecological assessment is needed for a riverine ecosystem, macroinvertebrates can be a proper start to understanding the general health of an area. Although we have been following macroinvertebrate collection and scoring methods derived by the Flint Watershed Coalition, these methods were developed for wadable streams and rivers. At our 3. Dam downstream site, macroinvertebrate collection methods were necessarily modified for non-wadable rivers which could have impacted scores at the downstream site over the course of this study.

Macroinvertebrates can be easily lost from the net when there are less than wadable conditions to deploy a net. For this reason, downstream macroinvertebrate scores are better compared temporally vs. spatially.

In conclusion, our data suggests that arsenic should be added to the contaminants monitored in the Michigan Eat Safe Fish Guides and more chemical contamination testing for water and sediment should take place to guide cleanup efforts for DDx, PCBs, arsenic, 1,4-dioxane, and vinyl chloride particularly at the 1. Non-urban downstream site, and 4. Dam upstream site as these sites had the most problematic sediment and water samples. The 4. Dam upstream site is particularly an area of concern due to the recent toxic waste spills, highly urbanized area, and as further restoration efforts could happen in this area and stir up contaminants from this site and allow them to contaminate other sites of the river. The knowledge of the contaminants in this area could raise discussion for cleanup efforts prior to any further restoration efforts at this stie. Fish tissue contaminants can prove difficult to aid in the understanding of ecological contamination of a specific area because fish move freely through the system, even more so now that the Hamilton Dam is removed. Furthermore, a replication of this data collection with improvements made to habitat mapping can provide important information on the long-term post-effects of the restoration event. Although environmental contaminants were taken into consideration by contractors when conducting the restoration event, it is not known if continuous data collection will be done on the river by those directly involved in the restoration event. Having long-term, comprehensive pre- and post- restoration data for urban low-head dams is an understudied area of ecological science but can prove important for maximizing the benefits of restoration efforts. The lessons learned from this case

study on the Flint River can be used to inform restoration strategies for any temperate urban river system constrained by a low-head dam.

This thesis highlights the ecological value of integrating aquatic community metrics, habitat assessments, water quality and contaminant analysis as a comprehensive assessment to assess the impacts of a century old urban low-head dam and inform future restoration efforts on riverine ecosystems. While fish tissue and sediment contaminant data revealed ongoing contamination concerns, especially for arsenic, PCBs, and VOCs like 1,4-dioxane, the aquatic and habitat findings also point to functional shifts driven by the presence of a dam in an urban setting. More importantly, this data provides a comprehensive pre-restoration baseline that will aid in the understanding of the post-restoration ecological effects of this large, long overdue restoration project on the Flint River.

Table II-1. Habitat assessment scores following the Michigan Department of Environmental Quality Procedure 51 for wadable and non-wadable streams as applicable (Michigan Department of Environmental Quality, 2013). This is a 100-point scale with scores indicating excellent (100-84), good (83-56), marginal (55-28) and poor (27-0). Macroinvertebrate scores are shown for spring and fall of 2023, and spring of 2024 following methods and scoring from the Flint River Watershed Coalition (FRWC) protocol with modifications to adapt to non-wadable rivers where applicable. The scale for this method is limitless, with scores indicating excellent (>48), good (48-34), fair (33-19) and poor (<19).

Site	2024 Habitat Score	2023 Spring Macro Score	2023 Fall Macro Score	2024 Spring Macro Score
1. Non-urban downstream	74 (Good)			42.9 (Good)
2. Urban downstream	59 (Good)			28.5 (Fair)
3. Dam downstream	21 (Poor)	2.1 (Poor)	7.2 (Poor)	9.3 (Poor)
4. Dam upstream	24 (Poor)	21.5 (Fair)	24.4 (Fair)	21.4 (Fair)
5. Urban upstream	49 (Marginal)			27.3 (Fair)
6. Non-urban upstream	72 (Good)			23.2 (Fair)

Table II-2. Of 30 models analyzed in AICc modeling, there were two top models that provided significance and the only models with a ΔAIC < 4 and Variance Inflation Factors > 6. Overall, Habitat assessment score was the only variable that provided a significant positive effect on macroinvertebrate score reflected in the 95% confidence intervals.

Explanatory Variable	**AICc Value**	**ΔAICc**	**K**	**Log likelihood**	**ω**
MacroScoreFRWC~ Arsenic + TEQPCB + Mercury + Habitat Assessment Score	87.9	0	3	-39.425	0.828
MacroScoreFRWC~ Arsenic + TEQPCB + Mercury + Habitat Assessment Score + Site Type	91	3.4	4	-38.638	0.172

Table II-3. Results of benthic sediment contamination concentrations with reference values. Reference values for Toxic Equivalency of Polychlorinated Biphenyls (TEQPCBs) was derived from the 2,3,7,8-TCDD pgTEQ/g referenced in the Canadian Sediment Quality Guidelines, 2002. The TEQPCB represents the cumulative toxicity of all PCB compounds found in each sample, as some compounds are more toxic than others. Perfluorooctanesulfonic acid (PFOS) does not have sediment quality guidelines, so no comparison can be made. Vinyl chloride and 1,4-dioxane were added as they are believed to be signature chemicals in the 2022 Lockhart Chemical spill. Reference values for vinyl chloride and 1,4-dioxane were derived from guidelines of rescinded U.S. EPA, Region 5 RCRA, Ecological Screening Levels (U.S. EPA, 2003). Values below the Method Detection Limit (MDL) were recorded as zero. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration.

*indicates a result above the reference value

Site	Replicate	TEQPCB (ppt)	PFOS (ppb)	1,4-dioxane (ppb)	Vinyl chloride (ppb)
Reference Value		21.5		119	202
1. Non-urban downstream	1	0.541	0.31	116	38.2
1. Non-urban downstream	2	0.627	0.41	104	33
2. Urban downstream	1	1.68	0.58	83.8	0.0016
2. Urban downstream	2	1.22	0.22	72.2	19.9
3. Dam downstream	1	0.269	0	62.5	16.1
3. Dam downstream	2	0.137	0	60.7	15.1
4. Dam upstream	1	55.4*	0	83.2	24.5
4. Dam upstream	2	115*	0	94.5	29.2
5. Urban upstream	1	0.0642	0	65.7	17.2
5. Urban upstream	2	0.0419	0.14	65.4	17.1
6. Non-urban upstream	1	0.007	0	64.4	16.1
6. Non-urban upstream	2	0.00084	0	63.1	16.1

Table II-4. Results of benthic sediment heavy metal contamination concentrations with reference values. Methylmercury (Hg), arsenic, cadmium, and lead reference values were derived from MacDonald Sediment Quality Guideline (SQG), specifically the consensus-based Probable Effects Concentrations (PEC). PEC's identify concentrations of contaminants that are above the harm threshold for sediment-dwelling organisms (Macdonald et al., 2000). Selenium values were referenced from the EGLE Water Resources Division Procedure 48. Values below the Method Detection Limit (MDL) were recorded as zero. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration. Values were used to caluculate Probable Effects Concentrations (PECq) for methods outlined in MacDonald 2000 to determine if values returned were statistically higher than the provided reference value and with high likelihood to cause harm effects to organisms. (indicated by asterisk).

*Statistically significant above the reference value using PECq

Site	Replicate	Hg (ppm)	As (ppm)	Cd (ppm)	Pb (ppm)	Se (ppm)
Reference Value		1.06	33	4.98	128	1.9
1. Non-urban downstream	1	0.062	11.8	0.87	63	1.1
1. Non-urban downstream	2	0.049	8.1	0.54	48.7	0.92
2. Urban downstream	1	0.18	10.3	1.3	163*	0.79
2. Urban downstream	2	0.015	13.7	0.18	15.4	0.8
3. Dam downstream	1	0.012	3.9	0.12	4.9	0.23
3. Dam downstream	2	0.012	3.2	0.12	4.1	0.22
4. Dam upstream	1	0.2	24.6	7.8*	186*	2.1*
4. Dam upstream	2	0.22	25	5.9*	533*	1.7
5. Urban upstream	1	0.012	2.9	0.12	4.4	0.23
5. Urban upstream	2	0.012	2.3	0.13	3.2	0.23
6. Non-urban upstream	1	0.011	2.6	0.12	1.2	0.22
6. Non-urban upstream	2	0.012	3.6	0.12	1.5	0.23

Table II-5. Concentrations for select contaminants in surface water samples. Threshold values were obtained from the National Recommended Water Quality Criteria – Human Health Criteria Table, using the "Organism Only" category. which indicates the health of the water assuming that people are not drinking the water, but still consuming fish from it over their lifetime. These thresholds are designed for average adults based on an average body weight and height. Any contaminant concentrations above these thresholds may pose a potential health risk to people who consume fish from the affected waters due to bioaccumulation. TEQPCB is listed in parts per trillion (ppt), where the remaining are listed in parts per billion (ppb). Contaminants shown include heavy metals, and persistent organic pollutants (e.g., PCBs, PFOS). The TEQPCB threshold (0.000064 ppb) applies to total PCBs, not toxic equivalency (TEQPCB). PFOS thresholds are from the EPA's 2024 draft human health criteria; 0.0000074 ppb represents the low end of PFOS concentrations observed in surface water globally. Lead is not regulated under these water quality criteria for human health; instead, it falls under the Lead and Copper Rule for drinking water. Michigan's lead and copper rule states an action limit for lead of 12 ppb. This action level signifies that if a home has multiple points in the home consistently testing over 12 ppb, the water is deemed unsafe for normal consumption. This value is reported in this thesis for reference only, as the Lead and Copper Rule takes into consideration the normal household drinking water consumption, and does not consider the biomagnification factor present in the environment. Values below the Method Detection Limit (MDL) were recorded as zero. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration. Statistical significance above the threshold value indicates that of the replicates at that site, greater than 10% of the measurements of water quality conditions exceed numeric reference value criteria, as stated by section 303(d) of the Clean Water Act.

Site	Replicate	TEQPCB (ppt)	PFOS (ppb)	As (ppb)	Pb (ppb)
Reference Value		0.064	0.0000074	0.14	
1. Non-urban downstream	1	0.00000444	3.7	4.7*	0
1. Non-urban downstream	2	0.00000583	0	4.8*	0
2. Urban downstream	1	0.00000245	4.5*	5.4*	0
2. Urban downstream	2	0.00000287	5.1*	5.6*	1.2
3. Dam downstream	1	0.00000308	2.5	5.3*	0
3. Dam downstream	2	0.00000345	0	5.2*	0
4. Dam upstream	1	0.000399	0	5.5*	0
4. Dam upstream	2	0.000164	5.5	5.6*	0
5. Urban upstream	1	0.0000399	0	5.2*	0
5. Urban upstream	2	0.00000122	0	5.1*	0
6. Non-urban upstream	1	0.000000855	0	5.2*	1.1
6. Non-urban upstream	2	0.000000816	0	5.2*	1.1

*Statistically significant above the reference value

Table II-6. Piscivore tissue contaminant concentrations from fish collected in 2024. Piscivores were tested for heavy metals due to the bio accumulative nature of the metals. Lead was removed from the figure as there were no levels of lead found above the Method Detection Limit (MDL). Levels for mammalian exposure were used for reference in this table. Values below the Method Detection Limit (MDL) were recorded as zero. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration.

Site	Replicate	Hg (ppb)	As (ppb)	Cd (ppb)	Se (ppb)
Oral Reference Dose (RfD)		.10	0.06	1	5
1. Non-urban downstream	1	0.46*	0	0	0.4
1. Non-urban downstream	2	0.22*	0	0	0.39
1. Non-urban downstream	3	0.12*	0	0	0.35
1. Non-urban downstream	4	0.079	0.12*	0	0.35
1. Non-urban downstream	5	0.18*	0.14*	0	0.33
1. Non-urban downstream	6	0.057	0.12*	0	0.34
2. Urban downstream	1	0.079	0.11*	0	0.38
2. Urban downstream	2	0.055	0.17*	0	0.31
2. Urban downstream	3	0.035	0	0.22	0.29
2. Urban downstream	4	0.32	0.18*	0	0.28
2. Urban downstream	5	0.03	0.23*	0	0.35
2. Urban downstream	6	0.12	0.13*	0	0.32
3. Dam downstream	1	0.2*	0.13*	0	0.37
3. Dam downstream	2	0.17*	0.15*	0	0.37
3. Dam downstream	3	0.11*	0.38*	0	0.26
3. Dam downstream	4	0.099	0	0	0.21
3. Dam downstream	5	0.19*	0.23*	0	0.26
3. Dam downstream	6	0.11*	0.15*	0	0.26
4. Dam upstream	1	0.12*	0.14*	0	0.27
4. Dam upstream	2	0.16*	0.17*	0	0.24
4. Dam upstream	3	0.069	0	0	0.2
4. Dam upstream	4	0.19*	0.22*	0	0.26
4. Dam upstream	5	0.1	0.16*	0	0.26
4. Dam upstream	6	0.14*	0.19*	0	0.29
5. Urban upstream	1	0.064	0.11*	0	0.18
5. Urban upstream	2	0.043	0	0	0.2
5. Urban upstream	3	0.041	0.17*	0	0.22
5. Urban upstream	4	0.036	0.11*	0	0.23
5. Urban upstream	5	0.1	0.12*	0	0.24

5. Urban upstream	6	0.055	0.14*	0	0.16
6. Non-urban upstream	1	0.44*	0	0	0.23
6. Non-urban upstream	2	0.12*	0	0	0.26
6. Non-urban upstream	3	0.087	0	0	0.3
6. Non-urban upstream	4	0.14*	0	0	0.28
6. Non-urban upstream	5	0.095	0.1	0	0.27
6. Non-urban upstream	6	0.32*	0	0	0.2

*Statistically significant above the reference value

Table II-7. Omnivores collected in 2024 with the added exception of rock bass were tested for perfluorooctane sulfonic acid (PFOS) and surrogates due to the nature of these contaminants and fish to reside throughout the water column. Zeros in the figure represent values that were below the Method Detection Limit (MDL). Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration.

Site	Replicate	PFOS (pg/L)
Reference Dose (RfD)		1000 pg/L
1. Non-urban downstream	1	1.72
1. Non-urban downstream	2	0.833
1. Non-urban downstream	3	0.222
1. Non-urban downstream	4	0.707
1. Non-urban downstream	5	1.34
1. Non-urban downstream	6	9.54
2. Urban downstream	1	14.5
2. Urban downstream	2	1.2
2. Urban downstream	3	25.8
2. Urban downstream	4	1.4
2. Urban downstream	5	0.557
2. Urban downstream	6	0.898
3. Dam downstream	1	0.0186
3. Dam downstream	2	0.961
3. Dam downstream	3	2.32
3. Dam downstream	4	1.25
3. Dam downstream	5	9.51
3. Dam downstream	6	3.16
4. Dam upstream	1	1.43
4. Dam upstream	2	1.72
4. Dam upstream	3	3.94
4. Dam upstream	4	0.494
4. Dam upstream	5	1.09
4. Dam upstream	6	0.349
5. Urban upstream	1	1.09
5. Urban upstream	2	0.254
5. Urban upstream	3	1.04
5. Urban upstream	4	1.4
5. Urban upstream	5	0.709
5. Urban upstream	6	0.428
6. Non-urban upstream	1	0.305
6. Non-urban upstream	2	1.43

6. Non-urban upstream	3	0.37
6. Non-urban upstream	4	1.82
6. Non-urban upstream	5	1.9
6. Non-urban upstream	6	0.203

Table II-8. Benthivore tissue contaminant concentrations for fish collected in 2024. Benthivores were tested for PCBs and DDT's and derivatives due to the nature of these contaminants to settle in sediment, and the nature of these fish to reside and feed close to benthic sediment. 2,4-DDD and 2,4-DDT were removed from the figure as there were no levels found above the Method Detection Limit (MDL). Zeros in the figure represent values that were below the MDL. Below the MDL does not mean zero, but so low that the machine used to test cannot accurately detect the concentration. TEQPCB indicates the Toxic Equivalency (TEQ) which is a value that represents the toxicity relative to all PCB compounds found in the sample (US EPA, 2012). RfD are not yet evaluated for DDE, DDD or PCBs. Oral Reference Doses were added for reference to data, from the USEPA Integrated Risk Information System. Oral Reference Dose is expressed in mg/kg-day and represents levels of ingestion that can cause certain toxicity effects on the body. RfD should be interpreted as consumption in ppb per day. (1 ppm = 1 mg/kg of contaminant and 1 ppb = 1 ug/kg). Mercury RfD is derived from the methylmercury source.

Site	Replicate	TEQPCB (pg/L)	2,4-DDE	4,4-DDD	4,4-DDE	4,4-DDT
Oral Reference Dose (RfD)						0.5 ppb
1. Non-urban downstream	1	1.72	0	0	0	0
1. Non-urban downstream	2	0.833	0	0	0	0
1. Non-urban downstream	3	0.222	0	0	0	0
1. Non-urban downstream	4	0.707	0	0	0	0
1. Non-urban downstream	5	1.34	0	0	0	0
1. Non-urban downstream	6	9.54	3.8	10.9	44.1	0
2. Urban downstream	1	14.5	5.1	8.5	56.4	6.8*
2. Urban downstream	2	1.2	0	6.2	106	16.3*
2. Urban downstream	3	25.8	12.2	29.1	152	41.3*
2. Urban downstream	4	1.4	0	0	0	0
2. Urban downstream	5	0.557	0	0	0	0
2. Urban downstream	6	0.898	0	0	0	0
3. Dam downstream	1	0.0186	0	0	0	0
3. Dam downstream	2	0.961	0	0	7.1	0
3. Dam downstream	3	2.32	0	0	8.4	0
3. Dam downstream	4	1.25	0	0	7.2	0
3. Dam downstream	5	9.51	0	23.6	72.4	0
3. Dam downstream	6	3.16	0	7.6	36.7	0
4. Dam upstream	1	1.43	0	0	0	0
4. Dam upstream	2	1.72	0	0	6.6	0

4. Dam upstream	3	3.94	0	0	14.7	0
4. Dam upstream	4	0.494	0	0	0	0
4. Dam upstream	5	1.09	0	0	0	0
4. Dam upstream	6	0.349	0	0	0	0
5. Urban upstream	1	1.09	0	0	8.5	0
5. Urban upstream	2	0.254	0	0	0	0
5. Urban upstream	3	1.04	0	0	17.3	0
5. Urban upstream	4	1.4	0	0	20.1	0
5. Urban upstream	5	0.709	0	0	8.1	0
5. Urban upstream	6	0.428	0	0	5.6	0
6. Non-urban upstream	1	0.305	0	0	0	0
6. Non-urban upstream	2	1.43	0	0	19.5	0
6. Non-urban upstream	3	0.37	0	0	9.8	0
6. Non-urban upstream	4	1.82	0	5.2	37.8	0
6. Non-urban upstream	5	1.9	0	0	25.9	0
6. Non-urban upstream	6	0.203	0	0	6.4	0

*Statistically significant above the reference value

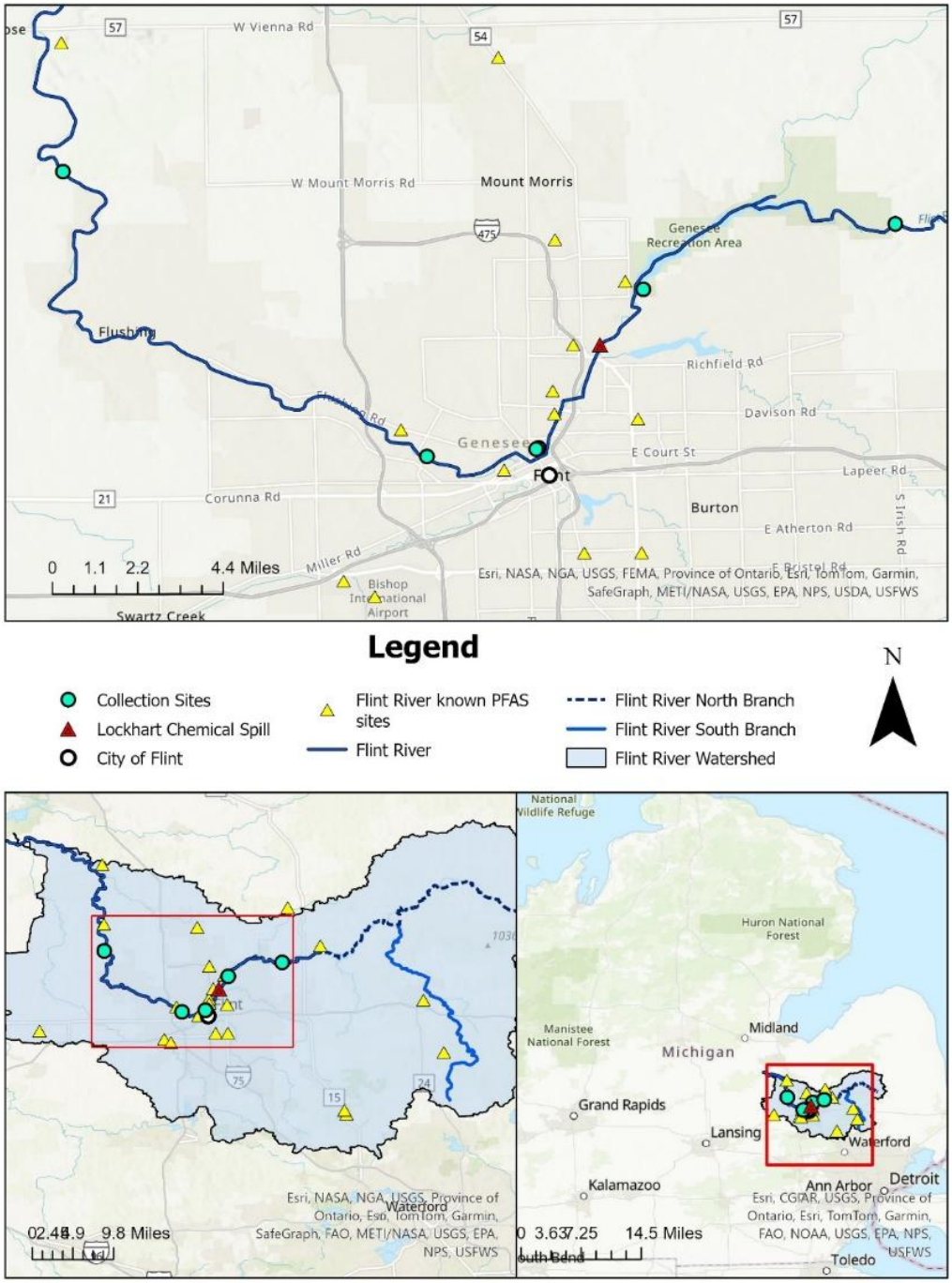


Figure II- 1. Map of known per- and polyfluoroalkyl substances (PFAS) presence sites, the chemical contamination collection sites, and location of the 2022 Lockhart Chemical spill in the Flint River Watershed Basin. Fish consumption guidelines are based off previous testing of fish tissues, the amount of safe consumption can vary depending on the weight and age of the person (US Food and Drug Administration, 2024).

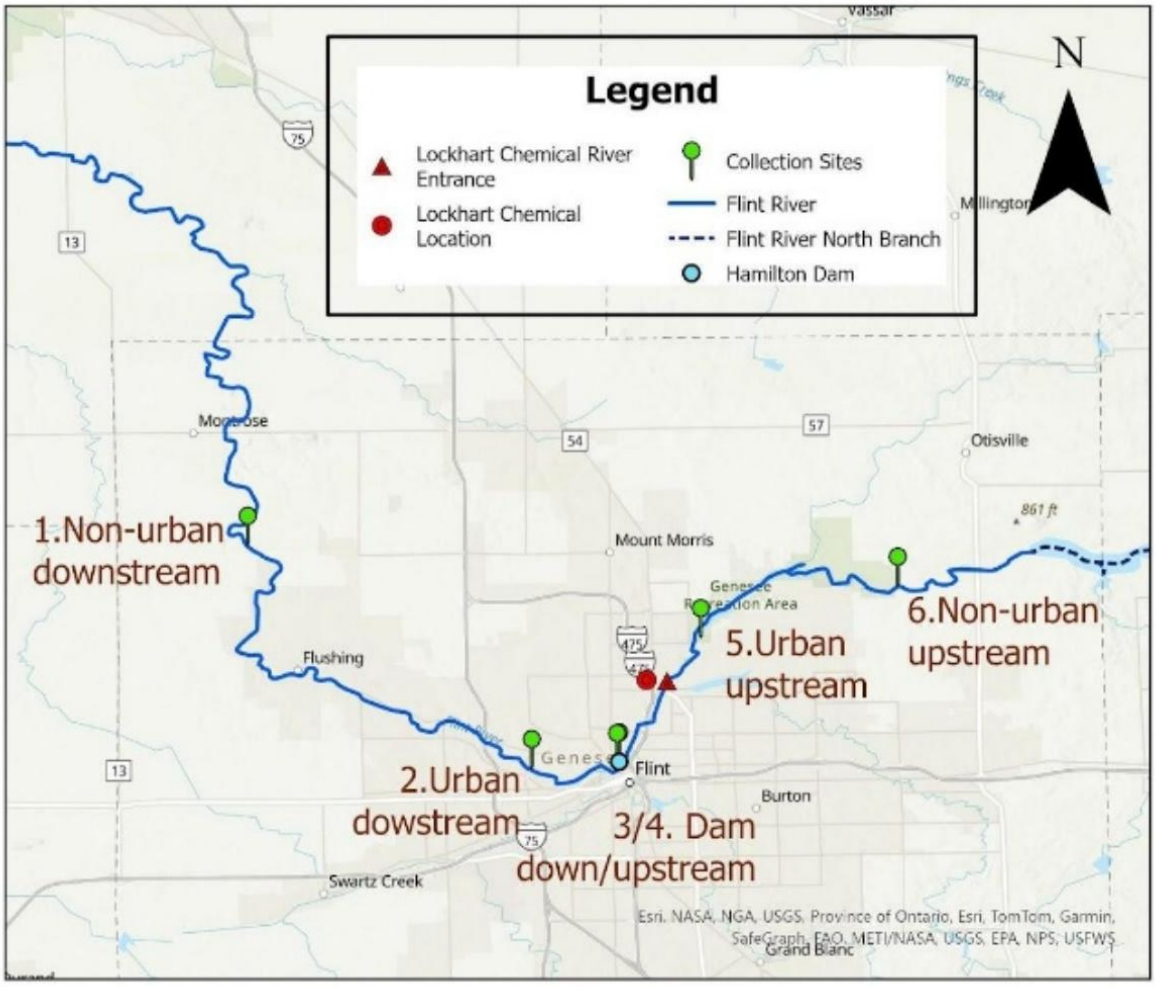


Figure II- 2. Chemical contamination collection sites along a gradient of anthropogenic disturbances along the Flint River, Michigan. Location of 2022 Lockhart Chemical spill and location of the building are included. The Hamilton Dam is located at 3/4. Dam down/upstream. The Holloway Dam is located ~700 river meters above the 5. Urban upstream site.

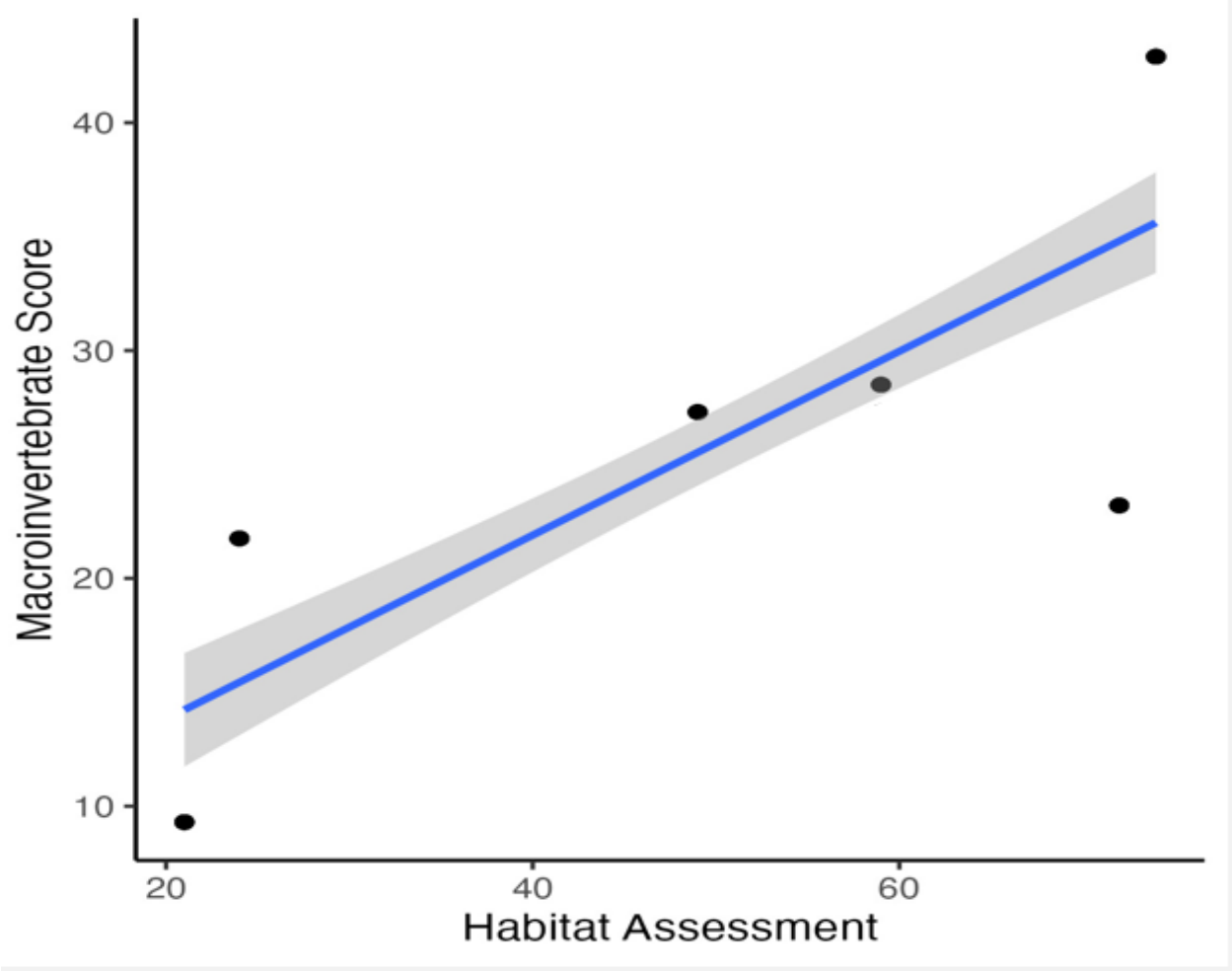


Figure II- 3. There was a positive relationship between macroinvertebrate collection scores following FRWC methods and scoring, and habitat assessments conducted following EGLE habitat assessment methods and scoring.



1. Non-urban downstream - Sediment: 1,4-dioxane, and Vinyl chloride were highest at this site although not above reference values	2. Urban downstream - Benthivores: Highest TEQPCBs, 4,4-DDE, and 4,4-DDT - Omnivores: PFOS highest - Water: presence of Pb	3. Dam downstream - Piscivores: contained the only fish that tested positive for levels of Cd - Benthivores: Second highest TEQPCBs and DDT's, second to urban downstream	4. Dam upstream - Arsenic: highest in fish, water and sediment. -Highest TEQPCBs in water and sediment - Sediment: All metals and TEQPCBs were highest at this site - Sediment: Cd, Pb, TEQPCB, all above reference values	5. Urban upstream	6. Non-urban upstream - Piscivores: Hg high - Surface water: Presence of Pb

Figure II- 4. Spatial distribution of key contaminant findings across six study sites along the Flint River, ordered from upstream (right) to downstream (left), following the direction of river flow. Sites include non-urban downstream (Flushing Township Nature Park), urban downstream (Mott Park), directly upstream and downstream of Hamilton Dam (3/4), urban upstream (Stepping Stone Falls), and non-urban upstream site (Irish Road Fishing Site). The visualization highlights spatial patterns in contaminant occurrence, including elevated PCBs, mercury, cadmium, arsenic, and lead in specific compartments for each site. For site 5. Urban upstream there were no negative conclusions to be gathered. In fact, this site had the lowest mean levels of metals in fish tissue.

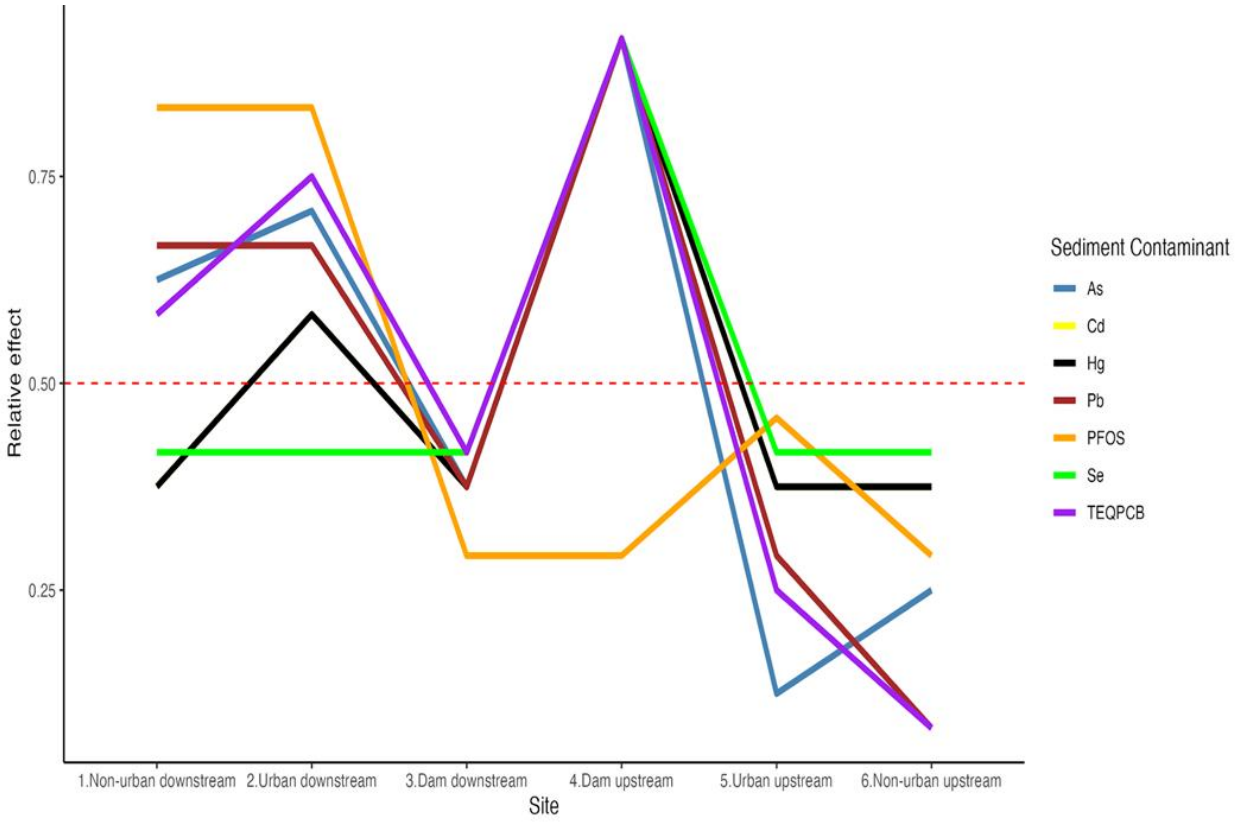


Figure II- 5. Relative effects graphs of sediment contaminants tested. Sediment samples were taken in duplicates at each of the 6 sites. Only contaminants with results are shown. Adjustments were made to remove contaminants with high multicollinearity from analyses (VIF > 6; 1,4-dioxane, vinyl chloride, DDx). The remaining variables in the model were significantly different between each other and between sites ($P < 0.05$). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites.

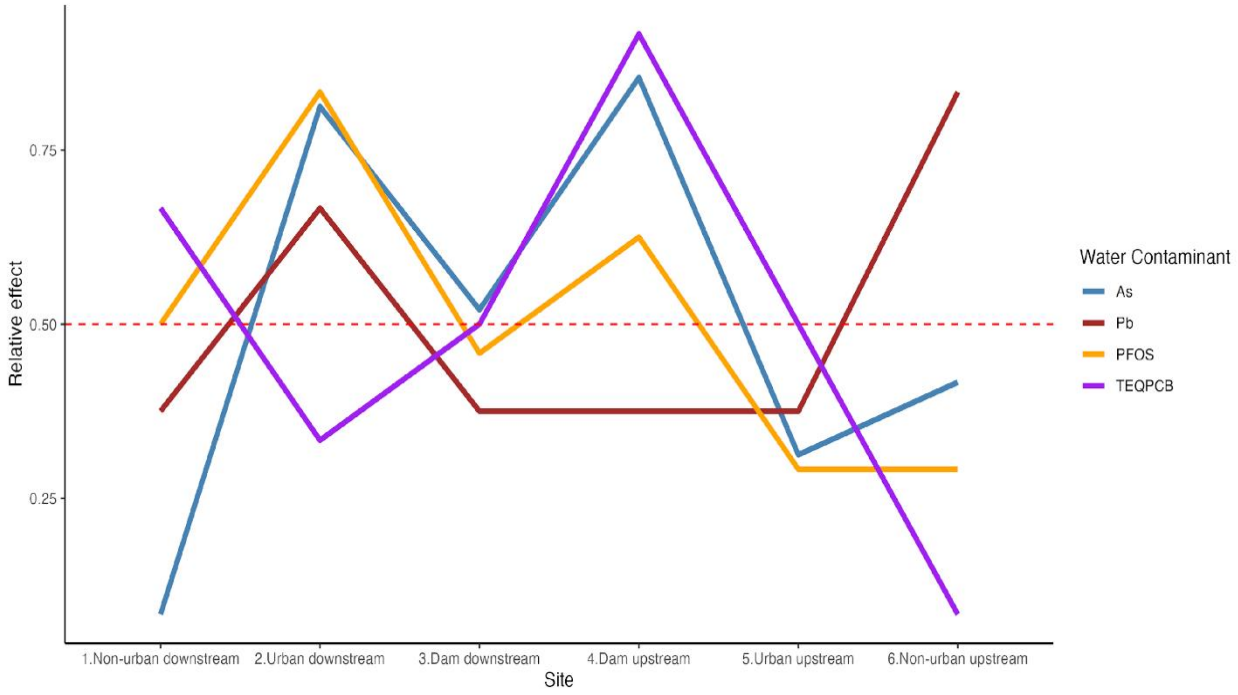


Figure II- 6. Relative effects graphs of surface water contaminants tested. Surface water samples were taken in duplicates at each of the 6 sites. Only contaminants with results are shown. Adjustments were made to remove contaminants with high multicollinearity from analyses (VIF > 6; 1,4-dioxane, vinyl chloride, DDx, mercury, cadmium, selenium). The remaining variables in the model were significantly different between each other and between sites (P =0.003). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites.

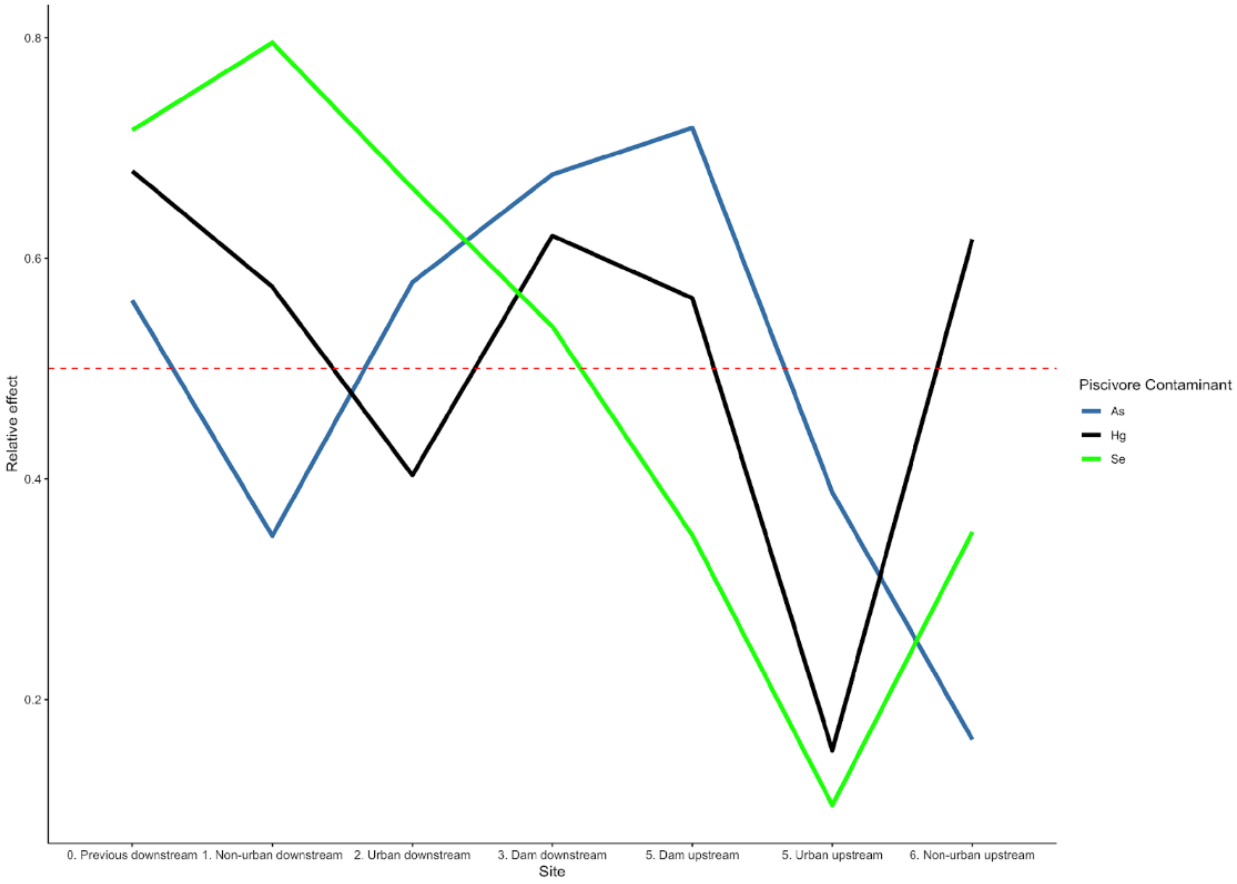


Figure II- 7. Relative effect graph for piscivore contaminants tested in 2024 and for fish collected at site 3. Dam downstream, prior to the toxic waste spills (indicated by 0. Previous downstream site). Piscivores were tested for As, Hg, Se, Pb, and Cd. Only contaminants with results are shown. Adjustments were made to remove contaminants with high multicollinearity from analyses (cadmium and lead). The remaining variables in the model were significantly different between each other and between sites ($P < .05$). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites.

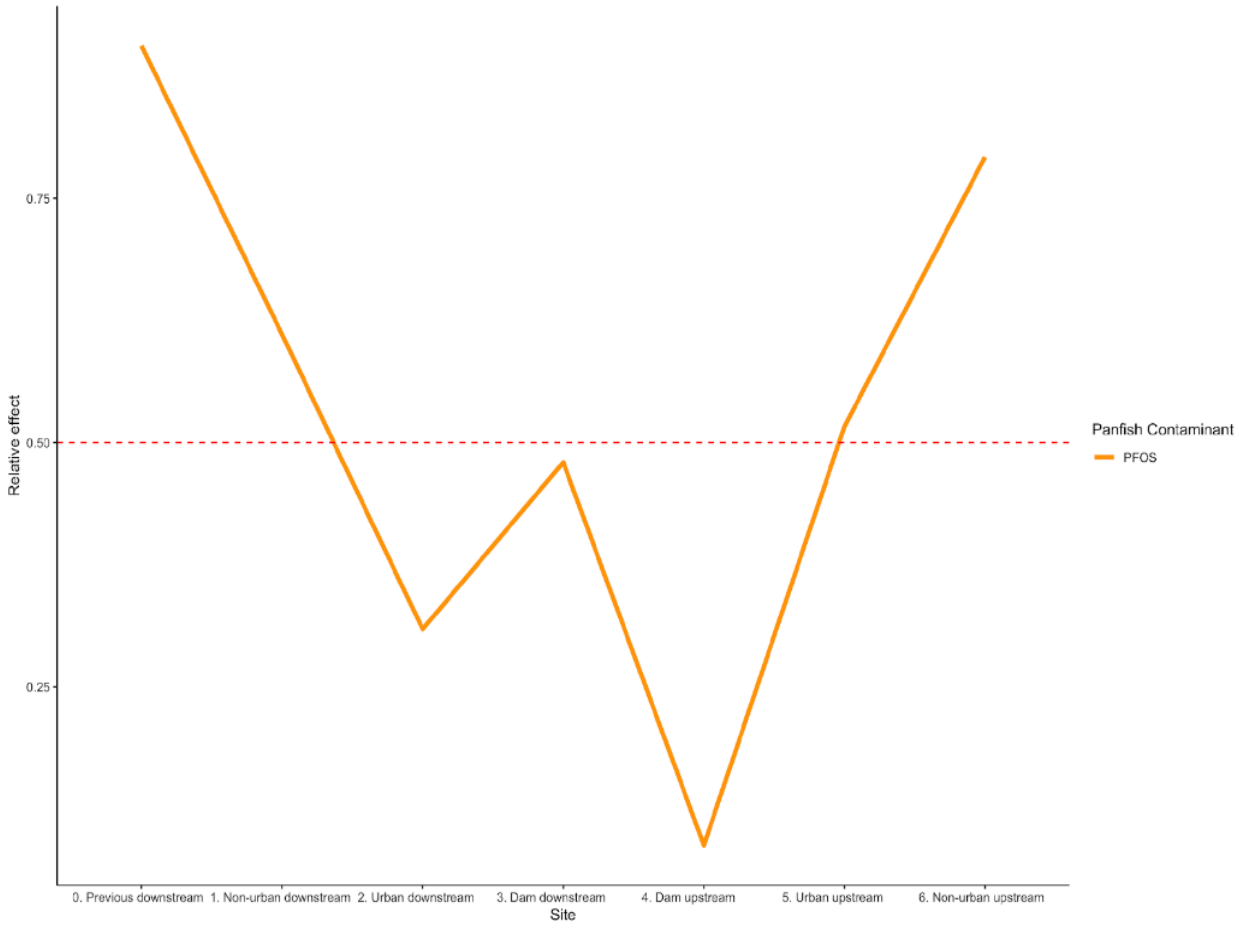


Figure II- 8. Relative effect graph for omnivore contaminants tested in 2024 and for fish collected at site 3. Dam downstream, prior to the toxic waste spills (indicated by 0. Previous downstream site). Omnivores (with the addition of rock bass) were tested for PFOS only. Statistical analysis reveals differences between the sites in contaminant levels ($P < 0.001$). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites.

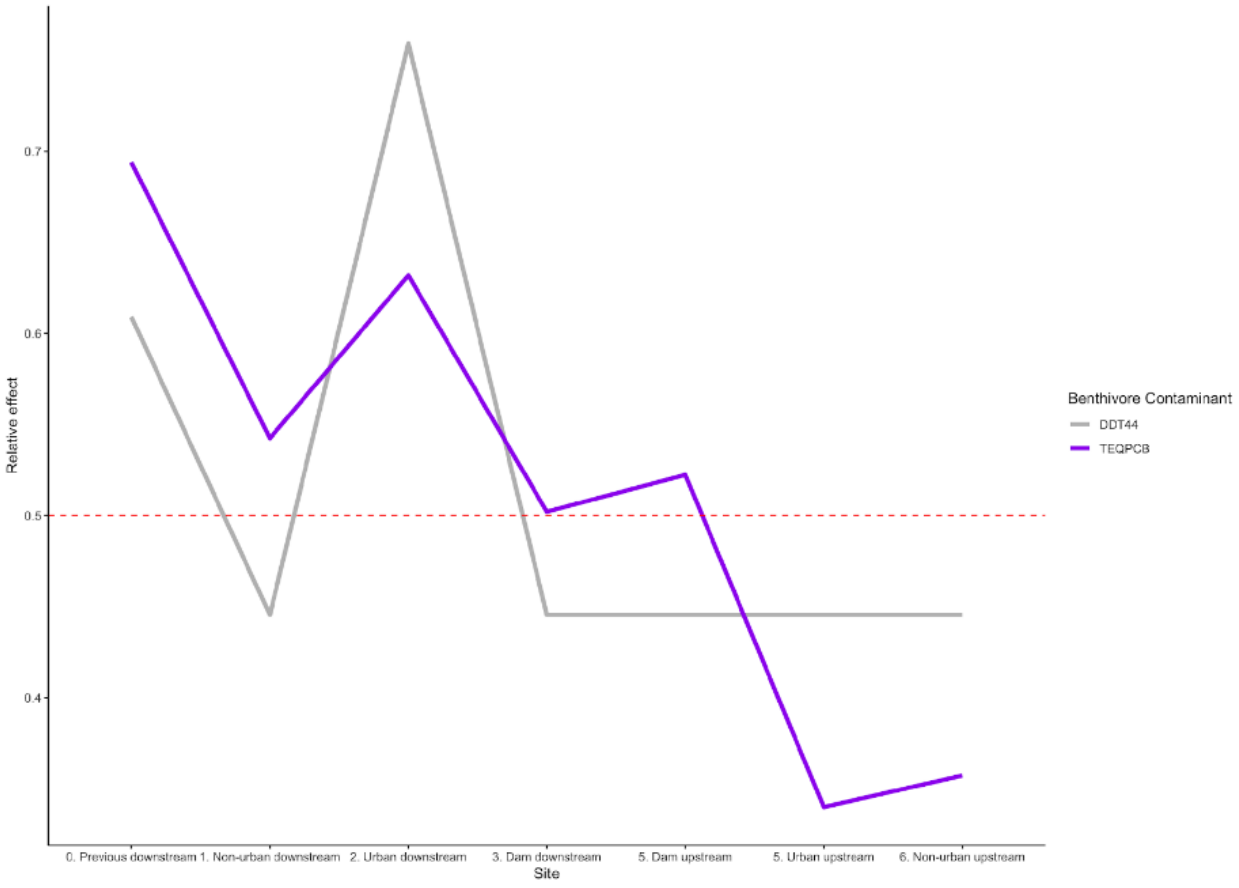


Figure II- 9. Relative effect graph for benthivore contaminants tested in 2024 and for fish collected at site 3. Dam downstream, prior to the toxic waste spills (indicated by 0. Previous downstream site). Benthivores were tested for the DDTs and derivatives and PCBs. Adjustments were made to remove contaminants with high multicollinearity from analyses (DDEs, 2,4-DDT). Statistical analysis reveals no differences between the sites in contaminant levels ($P > 0.05$). A value above the red dotted line (0.50) indicates that the contaminant was higher at that site than the compared sites.

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