EIGHTEENMILE CREEK AREA OF CONCERN

Degradation of Fish and Wildlife Populations Beneficial Use Impairment Removal Report

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Eighteenmile Creek Area of Concern

Degradation of Fish and Wildlife Populations

Beneficial Use Impairment (BUI) Removal Report

Prepared by:

New York State Department of Environmental Conservation

and

Niagara County Soil and Water Conservation District

This Beneficial Use Impairment (BUI) Removal Report was prepared by the New York State Department of Environmental Conservation (NYSDEC) and Niagara County Soil and Water Conservation District (NCSWCD) with substantial funding provided by the United States Environmental Protection Agency (USEPA) through the Great Lakes Restoration Initiative (GLRI). The NYSDEC and NCSWCD acknowledge the significant efforts of the Remedial Advisory Committee (RAC) in engaging stakeholders and the public throughout the BUI removal process. For more information, please contact either the Remedial Action Plan (RAP) Coordinator at NCSWCD or the Area of Concern (AOC) Coordinator at NYSDEC.

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List of Abbreviations

AOC	Area of Concern
BAP	Biological Assessment Profile
BUI	Beneficial Use Impairment
CPUE	Catch Per Unit Effort
EPT	Ephemeroptera, Plecoptera, Trichoptera
GLLA	Great Lakes Legacy Act
GLNPO	Great Lakes National Program Office
GLRI	Great Lakes Restoration Initiative
GLWQA	Great Lakes Water Quality Agreement
IJC	International Joint Commission
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
NCSWCD	Niagara County Soil and Water Conservation District
NOAEL	No Observable Adverse Effect Level
NYSDEC	New York State Department of Environmental Conservation
OU	Operable Unit
PCBs	Polychlorinated Biphenyls
RAC	Remedial Advisory Committee
RAP	Remedial Action Plan
RIBS	Rotating Integrated Basin Studies
TEQ	Toxic Equivalence
TSC	Toxic Screening Concentration
USACE	United States Army Corps of Engineers
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey

1. Introduction

This Beneficial Use Impairment (BUI) removal report identifies the background, criteria, supporting data, and rationale to remove the *Degradation of Fish and Wildlife Populations* BUI from the Eighteenmile Creek Area of Concern (AOC). The status of this BUI is currently designated as "Impaired" due to expected impact from contaminated water and bottom sediments. Yet, in recent years, several new studies have been completed to assess the extent to which contaminants in Eighteenmile Creek are impairing beneficial uses, including fish and wildlife populations.

The New York State Department of Environmental Conservation (NYSDEC) recommends the removal of the *Degradation of Fish and Wildlife Populations* BUI from the Eighteenmile Creek AOC, based on an evaluation of applicable data sets and evidence gathered to address this impairment. This recommendation is made with the full support of Niagara County Soil and Water Conservation District (NCSWCD) and the Eighteenmile Creek Remedial Advisory Committee (RAC).

2. Background

Under Annex One of the Great Lakes Water Quality Agreement (GLWQA), the International Joint Commission (IJC) has identified 43 AOCs in the Great Lakes Basin where pollution from past industrial production and waste disposal practices has caused significant ecological degradation. Up to fourteen BUIs are used as indicators of poor chemical, physical or biological integrity of the great lakes system that led to environmental degradation within an AOC.

Eighteenmile Creek flows through central Niagara County, New York from its headwaters in the Town of Lockport, to its discharge into Lake Ontario in Olcott approximately 18 miles east of the mouth of the Niagara River. The Eighteenmile Creek AOC includes the Olcott Harbor and extends upstream to the farthest point at which backwater conditions exist during Lake Ontario's highest monthly average lake level. This point is located just downstream of Burt Dam, approximately two miles south of Olcott Harbor (Figure 1).

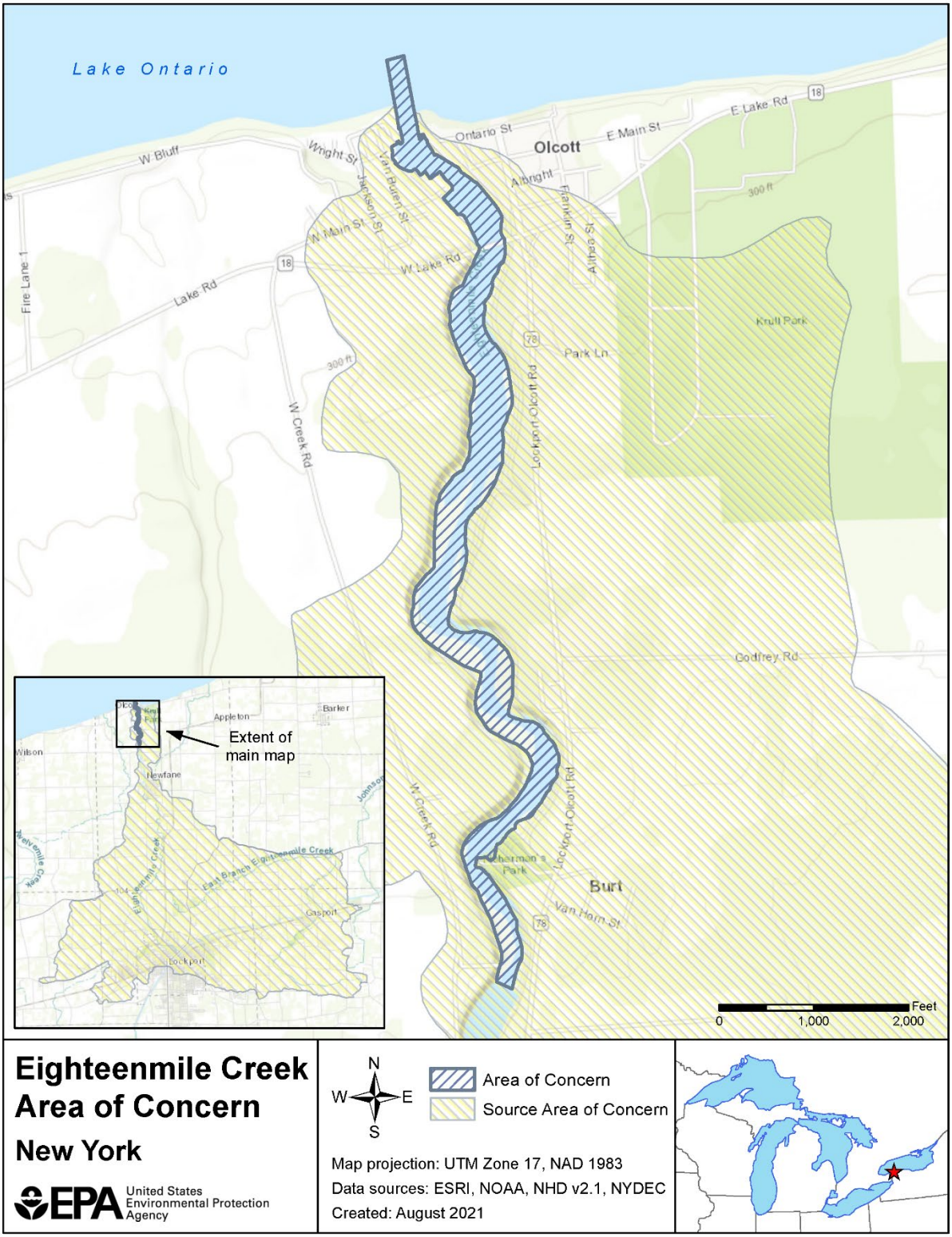

Figure 1 Eighteenmile Creek AOC boundary located in Niagara County, NY.

Eighteenmile Creek was designated as an AOC because water quality and bottom sediments were contaminated by past industrial and municipal discharge practices, the disposal of waste, and the use of pesticides. Over the years, numerous contaminants have been identified in creek sediments which have a detrimental effect to the AOC and Lake Ontario. As early as the 1997 Stage I/II Remedial Action Plan (RAP), the watershed upstream of the Eighteenmile Creek AOC, including the industrialized portions within the City of Lockport, have been identified as the likely source of contaminants impacting the AOC. The entire mainstem of Eighteenmile Creek, including upstream source areas and the AOC impact area, is now a designated site on the National Priorities List under the United States Environmental Protection Agency's (USEPA) Comprehensive Environmental Response, Compensation, and Liability Act (also known as Superfund).

Under Annex 1 of the GLWQA, AOCs are mandated to develop a RAP in three stages;

- Stage I, which collectively identifies specific BUIs and their causes,
- Stage II, which outlines the restoration work needed to address the root problems and restore the identified BUIs, and
- Stage III, which documents completion of these restoration activities and the delisting of the AOC.

Currently, the Eighteenmile Creek RAP consists of a combined Stage I/II RAP (NYSDEC, 1997) as well as several RAP updates. The most recent comprehensive Stage II RAP addendum was published in December 2011 (NCSWCD, 2011). The *Restrictions on Dredging Activities* BUI was removed in 2020 and the *Degradation of Fish and Wildlife Populations* is the second BUI proposed for removal.

2.1 Rationale for BUI Listing

The 1997 Stage I/II RAP presented an array of water quality monitoring and sediment sampling data that documented contaminant levels resulting in several BUIs being designated as impaired or 'impairment likely'. The BUIs listed as impaired in the Stage I/II RAP included *Restrictions on Fish and Wildlife Consumption*, *Degradation of Benthos*, and *Restrictions on Dredging Activities*. The status of several additional potential BUIs, including *Degradation of Fish and Wildlife Populations*, were initially classified as unknown.

The 2011 Stage II RAP Update changed the status of the *Degradation of Fish and Wildlife Populations* BUI to impaired based on an assumption that the elevated levels of polychlorinated biphenyls (PCBs) detected in fish flesh were likely causing negative effects on fish populations. It was noted in the Stage II update that bird and amphibian populations were likely not impaired and that there was insufficient data to determine whether mammal populations would be expected to be impaired (NCSWCD, 2011).

2.2 BUI Removal Criteria

To address the Eighteenmile Creek AOC BUIs, the RAC established restoration targets or "removal criteria" that determine when a BUI may be removed. Initial removal criteria for the *Degradation of Fish and Wildlife Populations* BUI were first introduced in the 2008 Delisting Targets Report (NCSWCD, 2008) and later included in the Eighteenmile Creek RAP Stage II Update (NCSWCD, 2011). In 2019, the NCSWCD and NYSDEC, in consultation with technical experts representing federal and state partner agencies including the United States Army Corps of Engineers (USACE), United States Fish and Wildlife Service (USFWS) and United States Geological Survey (USGS), evaluated the appropriateness of the removal criteria for each remaining BUI. Focus was placed on incorporating existing data and developing criteria which were

measurable, representative, and attainable for the region. As a result of these efforts, in 2020 the RAC approved modified removal criteria for all remaining BUIs. The final removal criteria for the *Degradation of Fish and Wildlife Populations* BUI are:

1. Fish community metrics (e.g., diversity, abundance, biomass, and condition) are similar to reference site(s); AND
2. Benthic macroinvertebrate community composition is within the range expected and similar to reference site condition; AND
3. PCB concentrations in fish tissue and other prey are below thresholds likely to result in acute toxicity to fish or piscivorous wildlife (birds and mammals).

The first criterion relies on direct measurement of AOC fish community metrics to determine if they are comparable with the regional reference condition. The second criterion uses macroinvertebrate community composition/condition as an indicator of a healthy ecosystem that would support robust fish and wildlife populations within the AOC. The third criterion requires consideration of potential lethal impacts due to PCB (or other contaminant) bioaccumulation in the AOC food web. Given the relatively small size of the Eighteenmile Creek AOC and surrounding watershed, the updated BUI removal criteria require a combination of direct and indirect assessment approaches. For example, fish and benthic macroinvertebrate communities can be sampled using relatively straightforward field-based approaches. Assessing wildlife populations presents more of a challenge due to the small size of the AOC, and the fact that many birds and mammals have food and home range sizes that extend beyond the AOC into neighboring streams or wetlands. As discussed in RAP documents, loss of fish and wildlife habitat is not believed to be an issue in the Eighteenmile Creek AOC. As such, the *Degradation of Fish and Wildlife Populations* BUI criteria are intended to determine if PCBs, metals, or other legacy pollutants are having population level impacts on fish and wildlife due to the toxicity of the water/sediment.

3. Monitoring and Assessments Supporting BUI Removal

3.1 Fish Community Studies

A 2007 BUI investigation concluded that fish populations were likely impaired due to contaminant impacts, namely PCB concentrations measured in brown bullheads that exceeded a toxic screening concentration (TSC) for chronic effects on fish (E&E, 2009, NCSWCD, 2011). To investigate if impacts were observed at the population level, whether due to sediment contamination or other causes, several fish community surveys have been conducted in the AOC and comparable reference areas.

To determine if fish communities in Eighteenmile Creek AOC are representative of regional reference conditions, fish communities were sampled in the AOC and in Oak Orchard Creek, a comparable stream that discharges into Lake Ontario approximately 26.5 miles to the east. Oak Orchard Creek is similar in surrounding geography to Eighteenmile Creek and also has a hydroelectric dam (Waterport Dam) near its mouth but is not known to have legacy chemical contamination (E&E, 2009). Oak Orchard Creek has also been determined to be a suitable reference site for other assessments including the *Degradation of Benthos* BUI and USEPA Superfund Program. A 2007 survey of fish communities in Eighteenmile Creek and reference sites in Oak Orchard Creek observed similar species composition and condition between both creeks, with a higher catch per unit effort (CPUE) in Eighteenmile Creek (E&E, 2009). In 2019, USGS and NYSDEC conducted an extensive monitoring effort to provide a more recent assessment of fish communities. The primary objective of this investigation was to evaluate the first BUI removal criterion

for the *Degradation of Fish and Wildlife Populations* BUI by determining if fish communities in the Eighteenmile Creek AOC are similar to regional reference areas. USGS sampled five reaches of Eighteenmile Creek AOC and Oak Orchard Creek using daytime boat electrofishing of nearshore habitats using standardized methods between the two creeks (Figure 2).

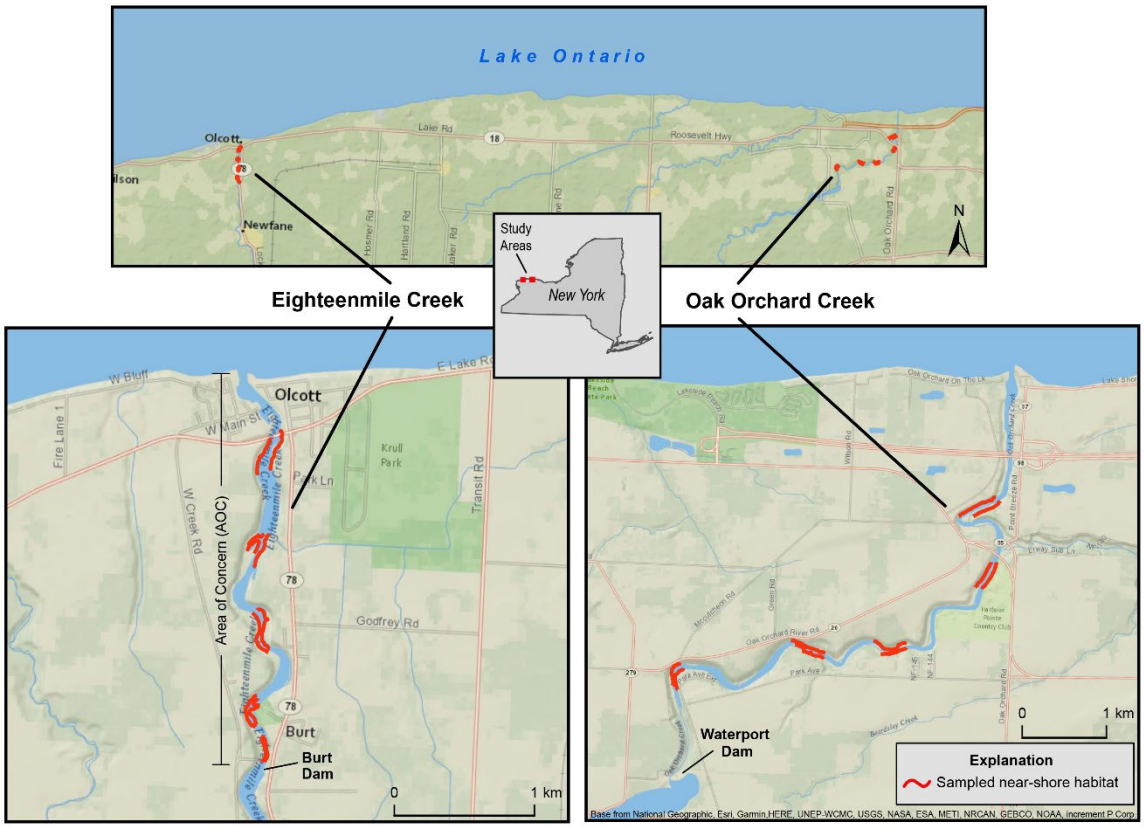

Figure 2: Location of reaches in the Eighteenmile Creek AOC and Oak Orchard Creek where fish communities were sampled (from George et al., 2022)

Estimates of fish community diversity, abundance, biomass, and fish condition from the sampled reaches of each creek were compared using a statistical testing framework (noninferiority tests demonstrating equivalence at the 95% confidence level). Noninferiority was established if there was 95% confidence that the test mean (AOC metric) was at least 75% of the reference mean (Oak Orchard Creek metric). A tolerance value of 25% was used because it has been recommended as appropriate for many environmental monitoring applications (Munkittrick et al., 2009). Diversity was measured using two indices (species richness and Shannon's Diversity Index), abundance was measured using a catch rate of fish per hour, and biomass was measured using a catch rate of mass (grams) of fish per hour. Fish condition was assessed for three common species (largemouth bass, bluegill, and brown bullhead) using a metric called relative weight. Relative weight is a measure of fish plumpness that uses the ratio of a fish's weight to an expected weight based on fish length at capture and is used as a surrogate for estimating fish health or condition (Neumann et al., 2012).

Statistical analyses indicated fish community diversity, biomass, and fish condition in the Eighteenmile Creek AOC were similar or superior to that in Oak Orchard Creek, while abundance was lower in the AOC

(Table 1, George et al. 2022). The difference in abundance between Eighteenmile Creek and Oak Orchard Creek was driven almost exclusively by the species golden redhorse (231 were captured in Oak Orchard Creek compared to 41 in Eighteenmile Creek).

Redhorse suckers are often considered to be sensitive to pollution and habitat degradation (Grabarkiewicz and Davis, 2008) and the golden redhorse New York State conservation status rank is listed as "Vulnerable" due to rarity or other factors including restricted range, and/or recent and widespread declines. However, golden redhorse were also present in all five subreaches on Eighteenmile Creek. In addition, the previous fish community surveys conducted during two seasons in 2007 found no evidence of impairment to abundance in the Eighteenmile Creek AOC (E&E, 2009).

Adding to the weight of evidence of no impairment to fish communities in Eighteenmile Creek, the other fish population metrics from the 2019 survey indicated greater fish community biomass and diversity (Shannon's Index of Diversity) and above average fish condition (relative weight) for largemouth bass and bluegill in Eighteenmile Creek. Thus, the weight of evidence from the 2007 and 2019 studies indicate the first removal criterion of the fish and wildlife populations BUI, "Fish community metrics (e.g., diversity, abundance, biomass, and condition) are similar to reference site(s)," has been met. The full suite of analysis and conclusions from the 2019 study are published in George et al., 2022 (provided as Appendix 1 of this BUI removal report) and the raw data are available in George, 2020.

Table 1 Key findings of the 2019 fish community survey including metrics of abundance, biomass, richness, diversity, and condition (relative weight of three common species). SE: standard error of the mean. Adapted from George et al., 2022. P-values ≤0.05 are bolded and indicate that noninferiority was established for Eighteenmile Creek.

	Eighteenmile Creek mean (SE)	Oak Orchard Creek mean (SE)	Noninferiority Test Result (P-value)
Mean Community Relative Abundance (fish/hour)	292 (69)	367 (68)	0.425
Mean Community Relative Biomass (grams/hour)	247,057 (34,078)	198,996 (41,079)	**0.035**
Richness (no. species)	18.0 (1.3)	18.6 (1.4)	**0.025**
Shannon's Diversity Index	2.47 (0.11)	2.18 (0.06)	**<0.001**
Relative weight of Largemouth Bass	105.9 (1.8)	100.4 (1.4)	**<0.001**
Relative weight of Bluegill	113.8 (2.0)	104.5 (1.3)	**<0.001**
Relative weight of Brown Bullhead	93.2 (1.8)	87.7 (2.3)	**<0.001**

3.2 Benthic Macroinvertebrate Community Surveys

Benthic assessments conducted between 1977 and 1994 in Eighteenmile Creek suggested macroinvertebrate communities were adversely affected by contaminated surficial sediments (NYSDEC, 1997, NCSWCD, 2011). These assessments, however, relied heavily on inferred or expected impact to benthic communities based on elevated contaminant concentrations in bed sediments. More recently, the Eighteenmile Creek AOC benthic macroinvertebrate community condition has been evaluated through surveys in 2014 and 2021, completed by USGS in conjunction with the NYSDEC and NCSWCD, within the AOC and non-AOC reference sites in Oak Orchard Creek. These datasets are relevant to the status of the second removal criterion for the *Degradation of Fish and Wildlife Populations* BUI which states "Benthic

macroinvertebrate community composition is within the range expected and similar to reference site condition". It was determined that comparing benthic communities in the Eighteenmile Creek AOC to those in the regional reference area (Oak Orchard Creek) would fully address this criterion as a community of similar condition to a reference area would thereby have a composition that was within the expected range (regional norm absent of contaminated sediments).

Macroinvertebrates were identified following NYSDEC Standard Operating Procedures (Duffy, 2021) to the lowest practical taxonomic resolution (usually genus). For each site, a measure of impact is assessed by averaging individual community metrics to yield a determination known as the Biological Assessment Profile (BAP) score. BAP scores incorporate multiple component metrics into a single value between 0 and 10 that is interpreted on a four-tiered scale of impact: severe (0.0–2.5); moderate (2.5–5.0); slight (5.0–7.5); or non-impacted (7.5–10.0). These BAP profiles provide a numeric ranking to assess a streams benthic macroinvertebrate community condition. Impact categories of moderate and severe are considered indicative of impaired conditions (Duffy, 2021). Estimates of benthic macroinvertebrate community condition (standard and aggregate BAP scores, George et al 2023) from each creek were compared using a statistical testing framework (noninferiority tests demonstrating equivalence at 95% confidence intervals). Aggregate BAP scores were calculated for the 2014 and 2021 surveys due to the inability to obtain the standard 100-organism count for each of the three replicates for each sampling location. For the aggregate BAP score approach, the data for the three replicates were combined to calculate BAP scores whereas the standard BAP score approach utilizes the individual replicates in calculation of the BAP scores for each sampling location.

The 2014 survey compared Eighteenmile Creek macroinvertebrate communities between the AOC (downstream of Burt Dam) and the Eighteenmile Creek upstream source area (above Burt Dam), with a similar sampling regime in Oak Orchard Creek by having samples collected upstream and downstream of Waterport Dam as reference sites. Analyses of benthic macroinvertebrate community integrity and structure indicated that macroinvertebrate communities, while impacted across most sites on both streams, were generally similar between the AOC and reference area. One site within the AOC, named emil-3, ranked poorly in most metrics. Since study results were somewhat inconclusive, additional data was needed to confidently determine macroinvertebrate community status (George et al. 2017).

To expand the Eighteenmile Creek macroinvertebrate dataset and gather additional evidence for assessment of the *Degradation of Benthos* BUI, a comprehensive assessment of macroinvertebrate community composition, community condition, and sediment toxicity in the AOC and reference sites was conducted in 2021. This survey consisted of eight sites within the AOC (downstream of Burt Dam) including all three AOC sites sampled in the 2014 survey, and six reference sites on Oak Orchard Creek downstream of Waterport Dam (Figure 3).

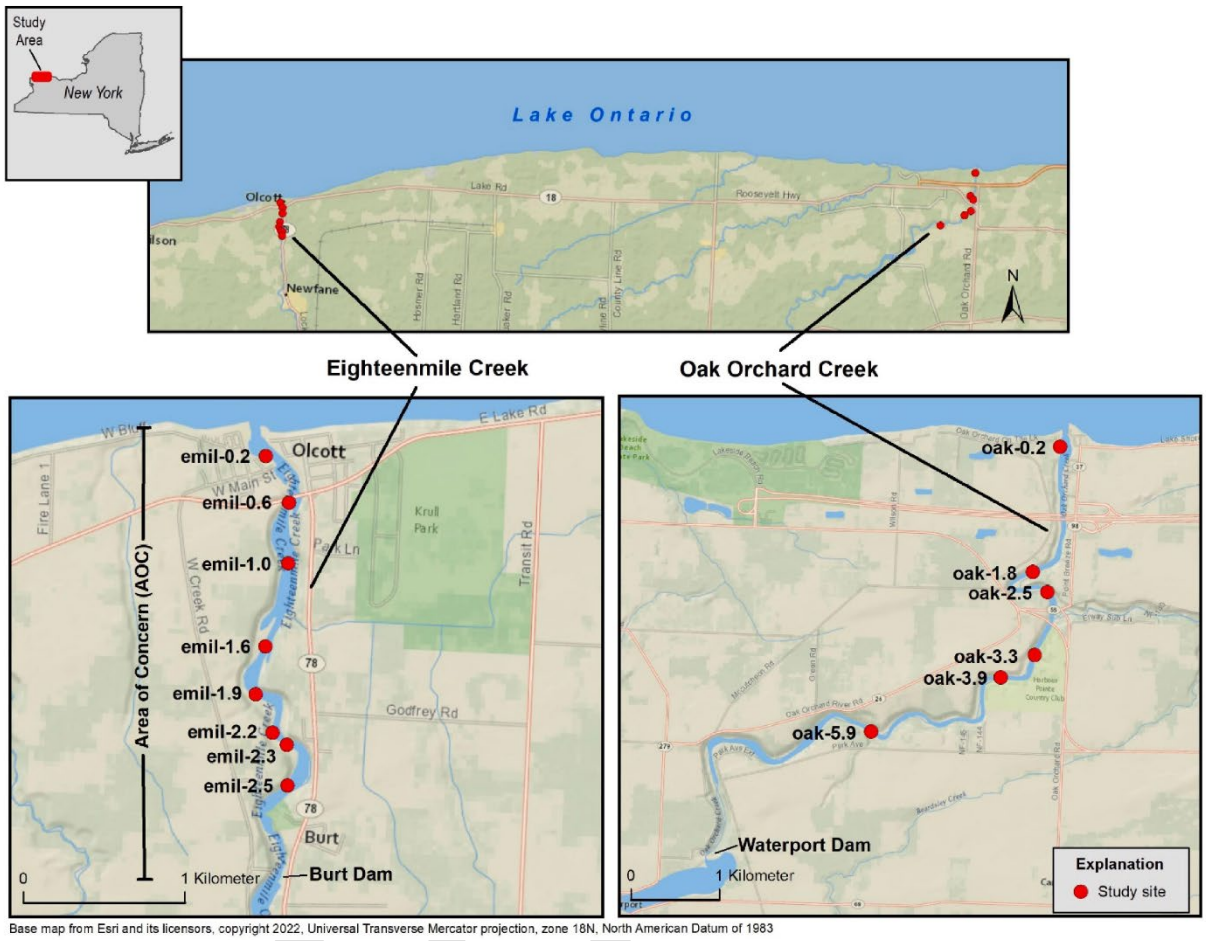

Figure 3: Location of benthic macroinvertebrate samples in the 2021 Eighteenmile Creek AOC and Oak Orchard Creek reference (from George et al., 2023)

Macroinvertebrate communities sampled in 2021 from the Eighteenmile Creek AOC were composed of organisms from 15 taxonomic orders and were dominated by chironomids in the order Diptera (primarily genera Procladius and Chironomus). Macroinvertebrate communities in the Oak Orchard Creek reference area were composed of organisms from nine taxonomic orders and were also dominated by chironomid-family Diptera (primarily genus Procladius). Eight orders were present in both the AOC and reference area, while seven orders were found exclusively in the AOC and one order (Coleoptera) was found exclusively in the reference area. The seven orders found only in the AOC include: Amphipoda, Basommatophora, Hoplonemertea, Lumbriculida, Megaloptera, Odonata, and Trichoptera. Within the sensitive EPT (Ephemeroptera, Plecoptera, Trichoptera) orders, Ephemeroptera (mayfly larvae) were present at six AOC sites, Plecoptera (stonefly larvae) were not found at any AOC site, and Trichoptera (caddisfly larvae) were present at two AOC sites (George et al 2023, Table 2). In the reference area, Ephemeroptera were found at the four upstream-most sites, while Trichoptera and Plecoptera were not found at any reference site.

The statistical results from the 2021 macroinvertebrate community assessment (George et al. 2023) indicated that the standard and aggregate mean BAP scores for the Eighteenmile Creek AOC (5.1 and 6.6, respectively, Table 2) were similar to the Oak Orchard Creek reference area (4.8 and 5.5, respectively,

Table 2). The Eighteenmile Creek standard mean BAP score (5.1) indicates the AOC as a whole is slightly impacted and the reference sites on Oak Orchard Creek standard mean BAP score (4.8) is moderately impacted.

Table 2 Key findings of the 2021 and 2014 macroinvertebrate community survey including standard and aggregate Biological Assessment Profile (BAP) score, presented as mean (standard error) and impact categories. The 2014 survey did not report standard error with BAP scores so only the mean is presented. Adapted from George et al., 2023 and George et al., 2017.

Site ID	Year	Standard BAP Score	Impact Categories	Aggregate BAP Score	Impact Categories
emil-0.2	2021	4.2 (1.3)	Moderate	6.5 (0.1)	Slight
emil-0.6	2021	4.1 (2.2)	Moderate	6.9 (0.1)	Slight
emil-1.0	2021	3.0 (0.7)	Moderate	4.6 (0.0)	Moderate
emil-1.6	2021	2.2 (0.4)	Severe	2.7 (0.1)	Moderate
emil-1.9	2021	7.2 (0.3)	Slight	7.51 (0.1)	Non-impacted
emil-2.2	2021	5.6 (0.6)	Slight	7.3 (0.0)	Slight
emil-2.3	2021	6.4 (1.7)	Slight	8.7 (0.0)	Non-impacted
emil-2.5	2021	8.1 (0.3)	Non-impacted	8.4 (0.0)	Non-impacted
oak-0.2	2021	3.9 (0.3)	Moderate	4.0 (0.1)	Moderate
oak-1.8	2021	4.9 (0.5)	Moderate	5.9 (0.0)	Slight
oak-2.5	2021	6.0 (0.1)	Slight	6.8 (0.0)	Slight
oak-3.3	2021	4.9 (0.4)	Moderate	5.6 (0.1)	Slight
oak-3.9	2021	3.9 (0.1)	Moderate	4.3 (0.1)	Moderate
oak-5.9	2021	5.4 (0.3)	Slight	6.4 (0.0)	Slight
emil-1	2014	4.9	Moderate	5.2	Slight
emil-2	2014	2.4	Severe	4.9	Moderate
emil-3	2014	2.1	Severe	3.9	Moderate
emil-4	2014	3.9	Moderate	6.4	Slight
emil-5	2014	5.4	Slight	7.5	Non-impacted
orch-1	2014	5.4	Slight	7.2	Slight
orch-2	2014	5.9	Slight	6.6	Slight
orch-3	2014	4.8	Moderate	6.1	Slight
orch-4	2014	4.8	Moderate	7.1	Slight
orch-5	2014	4.3	Moderate	6.0	Slight

Additionally, although not a focus of the removal criteria for this BUI, it is worth noting the 2021 survey also indicated the Eighteenmile Creek AOC macroinvertebrate community scores and sediment toxicity results may have met the *Degradation of Benthos* BUI removal criteria. The mean condition of macroinvertebrate communities, as calculated using both the standard BAP and aggregate BAP score, was 5.1 and 6.6 respectively, thus falling into the slightly impacted category and meeting the first criterion of

the *Degradation of Benthos* BUI of being '"non-impacted" or "slightly impacted" according to NYSDEC indices' (George et al 2023). There was also no evidence of sediment toxicity in 10-day toxicity tests with *Chironomus dilutus* and *Hyalella azteca* conducted at the same eight AOC sites.

Thus, the weight of evidence from the 2014 and 2021 surveys indicate the second removal criterion of the fish and wildlife populations BUI, "Benthic macroinvertebrate community composition is within the range expected and similar to reference site condition" has been met. The full suite of analysis and conclusions from each study is published in George et al., 2017 and George et al., 2023 (provided as Appendix 2 of this BUI removal report) and the raw data are available in George and Baldigo, 2022.

3.3 Wildlife Assessments

In 2007, two lines of evidence were examined to evaluate the potential impairment to wildlife populations (birds and mammals) by conducting seasonal wildlife population surveys within the AOC and Oak Orchard Creek (E&E 2009) and evaluating the risk of reproductive impairment to fish eating birds and mammals. Based on the 2007 results (E&E 2009) briefly summarized below, it was determined by the RAC that bird populations were not impaired, however, the limited and qualitative data available at that time was insufficient for evaluating the potential impairment of mammal populations and further assessment was recommended (NCSWCD, 2011). Monthly point count bird surveys conducted May through September 2007 and Marsh Monitoring Program bird surveys conducted in June within the AOC and Oak Orchard Creek indicated bird diversity and abundance of bird populations were similar (E&E 2009). For mammals, direct observations and signs of mammal presence were recorded coincident with the bird surveys and lower numbers of mammal species were observed at Eighteenmile Creek (9) when compared with Oak Orchard Creek reference sites (13). As noted in the final report (E&E 2009), the mammal observations provided qualitative information regarding mammal occurrences within the AOC and reference site. Overall, far fewer observations were made for mammals compared with birds due to the more secretive habits of many mammal species.

In the 2007 study (E&E 2009), Eighteenmile and Oak Orchard Creek brown bullhead total PCB and dioxins/furan concentrations were used to estimate exposure and risk for reproductive impairment to the great blue heron (*Ardea herodias*) and mink (*Mustela vison*), two wildlife species known to use Eighteenmile Creek and Oak Orchard Creek. The estimated exposure of the representative fish-eating bird (great blue heron) to total PCBs and dioxins/furans at Eighteenmile Creek was greater than at Oak Orchard Creek but did not exceed the lowest observed adverse effect level (LOAEL) for effects on bird reproduction at Eighteenmile Creek. Therefore, it was concluded bird populations were not impaired within the AOC (E&E 2009). The estimated exposure of the representative fish-eating mammal (mink) to total PCBs and dioxins/furans at Eighteenmile Creek was greater than at Oak Orchard Creek. For PCBs, the estimated exposure exceeded the LOAEL for effects on mammal reproduction at Eighteenmile Creek. Therefore, it was concluded there may be impairment to mammals (mink) from PCBs at Eighteenmile Creek (E&E 2009) and further investigations were recommended.

In 2010, USACE adapted a *TrophicTrace* foodweb model that used measured concentrations in sediment and fish to estimate PCB bioaccumulation in birds (great blue heron and belted kingfisher), fish, and American mink. Fish were collected from Eighteenmile Creek to compare actual tissue concentrations to modeled results and used to refine the model for better accuracy. Birds showed no or low potential for risk of harmful effects, indicating likely no impairment (E Risk Sciences and USACE ERDC, 2012). Mink had low potential for risk of harmful effects, however, it was noted that there was uncertainty due to overlap

between no observable adverse effect levels (NOAELs) and LOAEL (i.e. the highest NOAEL exceeded a LOAEL). While these results provided valuable insight into potential PCB impacts to birds (no impairment), the RAC decided another study was needed to assess any possible effects to mammals (mink in particular) due to known mink sensitivity to PCBs.

As a result, an additional assessment focused on mink was conducted by the College at Brockport, State University of New York (SUNY Brockport) starting in 2018 (Haynes and Wellman, 2022; provided as Appendix 3 of this BUI removal report). Mink was chosen as an indicator species because they consume mostly aquatic organisms and are sensitive to PCB-related toxicity. Previous research has shown that mink populations are especially sensitive to dioxins, furans, and dioxin-like PCBs. The most sensitive biomarkers of effect from these chemicals include cancerous jaw lesions and reproductive failure. The purpose of this study was to document whether mink consuming a diet with high proportions of aquatic prey from the Eighteenmile Creek AOC and Oak Orchard Creek reference area would accumulate concentrations of chemicals of concern (such as PCBs, mercury, dioxins and furans) high enough to cause acute (lethal) or chronic (health) effects , in support of evaluation of the *Degradation of Fish and Wildlife Populations* and the *Bird or Animal Deformities or Reproductive Problems* BUIs.

SUNY Brockport's approach included the use of both a diet model and a bioaccumulation model to predict mink dietary concentrations of total mercury, total PCB and PCB, dioxin, and furan toxic equivalents (TEQ). TEQ is a better measure of toxicity to wildlife than total PCB (Giesy and Kannan 2002) as TEQ assesses the toxic potency of each compound relative to dioxin. The Eighteenmile Creek PCB mixture (predominantly Arochlor 1248, Pickard as cited in Haynes and Wellman, 2022) is composed of less toxic PCB compounds than Acochlor 1254 or dioxin. Model outputs were compared to each other, and to literature-based lowest observable adverse effects concentrations (LOAECs), to assess possible acute and chronic hazards to mink.

Overall study results indicate that concentrations of total mercury and dioxin/furan TEQ in the study area are not of biological concern to mink. Total PCB and PCB TEQ concentrations in mink prey, namely different trophic levels of fish, are an order of magnitude higher in the Eighteenmile Creek AOC than in the Oak Orchard Creek reference site. The Eighteenmile Creek upper trophic level fish total PCB concentrations did exceed the acute LOAEC for Arochlor 1254, however, PCB TEQ in upper trophic level fish were below the acute Arochlor 1254 LOAEC. As noted previously, the lesser toxic PCB mixture, Arochlor 1248, is the predominate PCB mixture in the AOC and the PCB TEQ represents the toxic potency of the PCB mixture as compared to the most toxic compound (dioxin) while total PCB is a sum of all PCB compounds (toxic and less toxic) regardless of toxic potency compared to dioxin. In addition, mink diet does not exclusively consist of upper trophic level fish.

The mink diet and bioaccumulation exposure prediction models for total PCB and PCB TEQ were in agreement with exposure and risk estimates. The Eighteenmile Creek mink prey diet models for total PCB and PCB TEQ did not exceed the acute dietary LOAECs, which are relevant to the *Degradation of Fish and Wildlife Populations* BUI. Total PCB and PCB TEQ did exceed the chronic dietary LOAECs, which are relevant to the *Bird or Animal Deformities or Reproductive Problems* BUI. Even with elevated risk (exceedance of acute Arochlor 1254 LOAEC for total PCB in upper trophic level fish) from consuming high proportions of aquatic prey in the Eighteenmile Creek AOC, mink would be highly unlikely to suffer acute toxicity from PCBs based on the dietary model predictions. Dietary risk exposure estimates including data from all trophic level prey groups predict total PCB concentrations would fall below the Arochlor 1254 LOAEC

(Haynes and Wellman, 2022). It is important to note, the *Degradation of Fish and Wildlife Populations* BUI removal criteria specifically identify acute toxicity thresholds. Therefore, findings from the SUNY Brockport study related to chronic effects are not addressed in this BUI removal report. Thus, the weight of evidence from the 2007, 2010, and 2018 studies indicate the third removal criterion of the fish and wildlife populations BUI, "PCB concentrations in fish tissue and other prey are below thresholds likely to result in acute toxicity to fish or piscivorous wildlife (birds and mammals)" has been met.

4. Sediment Management Actions

Historic investigations of the Eighteenmile Creek AOC have not identified significant sources of legacy contaminants originating from within the AOC boundaries. This has led to a significant amount of work completed by federal, state, and local partners to identify, characterize, and delineate upstream sources of contamination to Eighteenmile Creek. Extensive sediment sampling completed by the Great Lakes Legacy Act (GLLA) confirmed contaminants such as PCBs and metals exceeding state and federal superfund sediment guidance values. These contaminants were traced to source areas upstream of the AOC, including the Eighteenmile Creek Corridor, a 4,000-foot section of the creek and associated upland areas that span from the NYS Barge Canal to Harwood Street in the City of Lockport.

4.1 USEPA Superfund Site

Due to the extent of contamination in Eighteenmile Creek source areas and associated cost of remediation, the NYSDEC requested the Eighteenmile Creek Corridor and stream channel sediments be nominated to USEPA's Superfund program. The entire length of the creek, from the NYS Barge Canal in Lockport to the outlet at Lake Ontario approximately 15 miles downstream in Olcott, New York was placed on the National Priorities List, or Superfund, in 2012. The main contaminants of concern are lead and PCBs.

The Superfund program divided the Eighteenmile Creek cleanup into four different Operable Units (OUs) based on the type of work and geographic area. The first phase of the cleanup (OU1) included the demolition of the former Flintkote factory, which was a likely source of PCBs and metals to Eighteenmile Creek. This first step of the cleanup (OU1) was completed in 2016. The second phase of the cleanup (OU2) involves a combination of excavation and capping of contaminated sediment and soil, respectively, within the Creek Corridor. A record of decision for OU2 was issued in 2017, with remedial work expected to begin as early as 2024. OU3 includes the restoration of groundwater in the Creek Corridor as well as creek sediments and floodplain soils from where OU2 ends (near Harwood Street in Lockport) to where the creek discharges into Lake Ontario (Figure 4). OU3 is still under investigation by the Superfund program to determine appropriate and cost-effective cleanup options. OU4 involves removing lead-contaminated soil at residential properties adjacent to the former Flintkote property in the City of Lockport. A record of decision for OU4 was issued in 2018, with cleanup beginning in 2024.

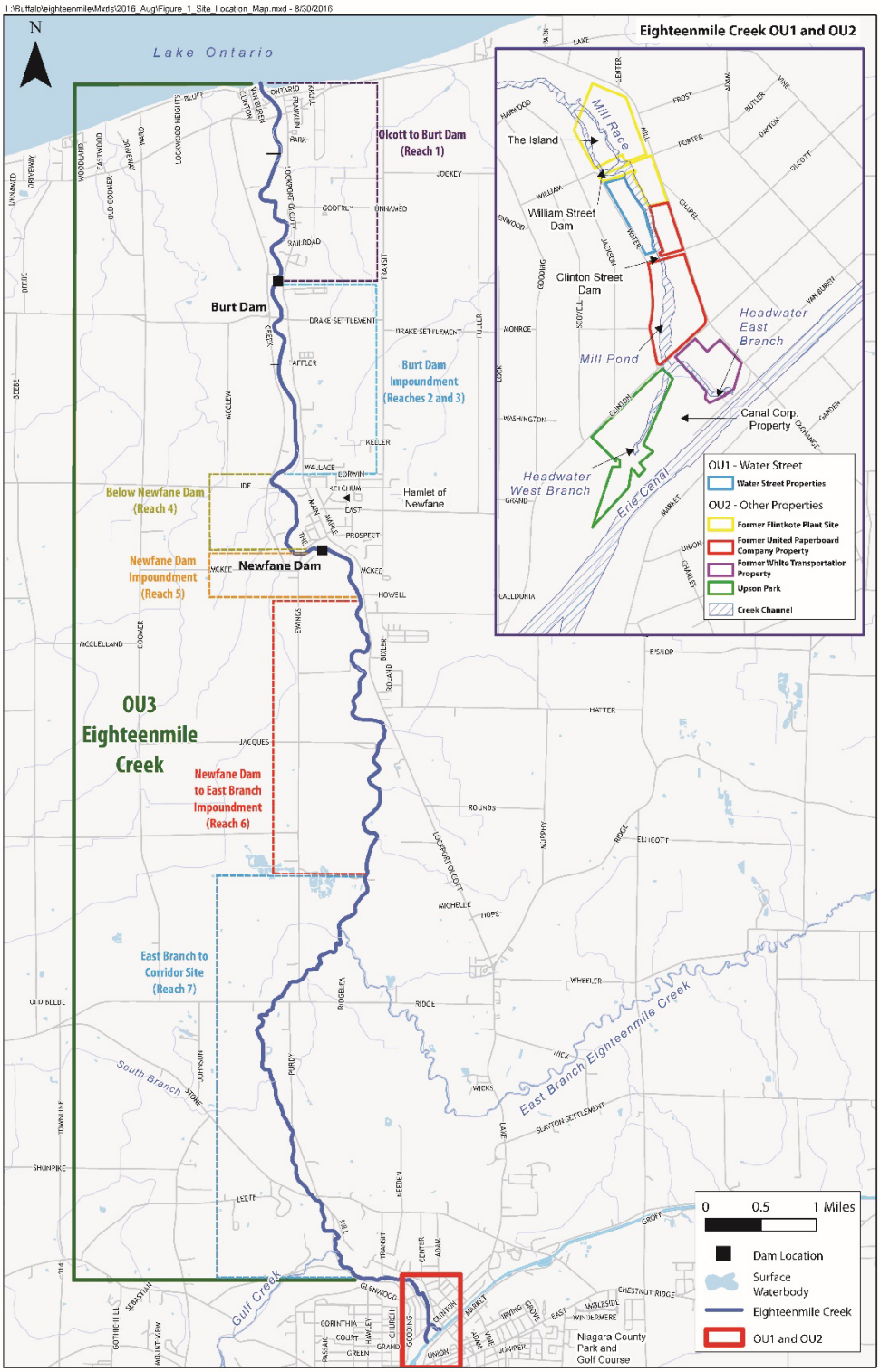

Figure 4: Operable Unit overview map showing the locations of Operable Units 1,2 and 3. Eighteenmile Creek AOC comprises Reach 1 of OU3. (from USEPA, 2017)

4.2 Great Lakes Restoration Initiative Management Actions

In January 2020, the USACE completed a desktop review of available sediment chemistry data for the Eighteenmile Creek AOC (Pickard et al 2020). One of the primary objectives of this review was to determine if any sediment quality-related Management Actions are necessary to remove the BUIs for the AOC. The USACE evaluation of the benthic macroinvertebrate community data conducted in 2019 (Pickard et al., 2020) noted that poor benthic results at a single site (during the 2014 survey) should not be considered unusual and may not be related to contaminant levels in sediment. Based on a review of all available benthic data through 2019, USACE concluded that no such sediment-related Management Actions were necessary within the AOC to address the BUIs (Pickard et al., 2020). This interpretation was subsequently supported by the findings from the 2021 benthic sampling which found no abnormal results at the site that scored poorly in 2014 (or at any other AOC site).

In the context of the Great Lakes Area of Concern program, a "Management Action" is defined as a key Great Lakes Restoration Initiative (GLRI) or GLLA funding commitment for a major project or strategic set of projects intended to bring about significant restoration of water quality and water dependent resources, consistent with the GLWQA. The primary examples of GLRI/GLLA Management Actions for AOCs typically fall into the general categories of water pollution source control, contaminated sediment remediation, and habitat restoration. Under this definition, assessments, and monitoring projects to evaluate the status of BUIs are not considered to be Management Actions. Major habitat restoration, source control and sediment remediation or sediment maintenance initiatives under separate federal, state, or local programs (such as the federal Superfund program) are not considered to be Management Actions. Based on the recommendations made by the USACE, as well as the collective efforts achieved to date by local, state, and federal partners, Eighteenmile Creek AOC was designated as Management Action Complete in 2020. This determination was made by NYSDEC with support from NCSWCD and the Eighteenmile Creek RAC, and concurrence from USEPA.

The AOC program is collaborative and many of the goals outlined in the Eighteenmile Creek Stage I/Stage II RAP are contingent upon the completion of remedial projects through federal Superfund programs. The Eighteenmile Creek AOC comprises Reach One of OU3 of the Eighteenmile Creek federal Superfund site. It is imperative that pertinent planning, design, implementation, and ultimate completion of the Superfund remedies in and around Eighteenmile Creek support BUI restoration targets to the greatest extent possible. While recent monitoring and assessment data may support the removal of some BUIs at this time, completion of the Superfund remedies will be necessary to ensure the full implementation of the Eighteenmile Creek RAP, removal of other BUIs, and ultimately delisting of the AOC.

5. Public Outreach

NYSDEC, in partnership with NCSWCD, USEPA, and the Eighteenmile Creek RAC, hosted a public meeting on _____to present the case for removing the *Degradation of Fish and Wildlife Populations* BUI to local stakeholders. The meeting was held during the 30-day period from _____ during which public was invited to review and provide input on a draft version of this BUI removal report, which was hosted on the Eighteenmile Creek RAPs website.

(Placeholder/Possible Appendix 4 for summary of public engagement process, to be prepared after the 30-day period noted above.)

6. Conclusions

6.1 Removal Statement

In the Stage II RAP Update for the Eighteenmile Creek AOC, the *Degradation of Fish and Wildlife Populations* BUI was listed as impaired due to the concentrations of PCBs in fish (NCSWCD 2011). While tissue concentrations in fish are elevated when compared to reference areas, there is no evidence that these concentrations are degrading fish populations, benthic macroinvertebrate communities, nor bioaccumulating in fish, birds, or mammals that would result in acute (lethal) toxicity. Remedial efforts completed by USEPA Superfund are expected to reduce upstream sources further improving the quality of sediments in Eighteenmile Creek including the AOC.

The BUI removal criteria state "1. Fish community metrics (e.g., diversity, abundance, biomass, and condition) are similar to reference site(s); AND 2. Benthic macroinvertebrate community composition is within the range expected and similar to reference site condition; AND 3. PCB concentrations in fish tissue and other prey are below thresholds likely to result in acute toxicity to fish or piscivorous wildlife (birds and mammals)."

Multiple lines of evidence discussed in this report indicate the removal criteria are met to redesignate the BUI as not impaired. In summary, fish populations within the AOC section of Eighteenmile Creek (below Burt Dam) are similar or superior to reference sites at Oak Orchard Creek. Biomass, diversity, and fish condition were all similar or superior in Eighteenmile Creek when compared to Oak Orchard Creek (criterion 1). Benthic macroinvertebrate communities across Eighteenmile Creek AOC are largely comparable to Oak Orchard Creek and any degradation in community condition is not caused by contaminants identified in the Eighteenmile Creek RAP but by regional conditions including sedimentation, poor habitat, or seasonal eutrophication (Pickard et al 2020, George et al. 2023). Despite some level of impact according to DEC BAP metrics, benthic macroinvertebrate communities are similar to Oak Orchard Creek and are intended to serve as an ecosystem indicator supporting wildlife populations (criterion 2). Wildlife contaminant modeling in the Eighteenmile Creek AOC for PCBs and other contaminants of concern suggest that acute impacts are unlikely (criterion 3).

Although remedial action is ongoing in the Eighteenmile Creek watershed, BUI assessments have shown removal criteria are met and the NYSDEC has determined the *Degradation of Fish and Wildlife Populations* BUI can be removed from the list of designated impairments for the Eighteenmile Creek AOC in accordance with USEPA guidance and the GLWQA. The Eighteenmile Creek RAC and NCSWCD fully support the removal of this BUI.

6.2 BUI Removal Steps

	Completed	*Date*	*Step Taken*
1.	✓	10/2008	BUI first designated as "impaired" in a delisting target report to USEPA.
2.	✓	05/2019	Final BUI removal criteria established with RAC consensus.
3.	✓	01/2022	RAC agreed to proceed with BUI removal.
4.			Initial Draft BUI removal recommendation provided to USEPA Technical Review Lead.
5.			Receive comments from USEPA Technical Review Lead and revise removal report accordingly.
6.			Hold public outreach meeting to present BUI removal rationale to local stakeholders (including a 30-day public comment period).
7.			NCSWCD/NYSDEC completes final modifications to the *Degradation of Fish and Wildlife Populations* BUI removal document, based on public comments received.
8.			Coordinate the formal transmittal of the BUI removal report with USEPA Great Lakes National Program Office (GLNPO).
9.			Communicate results to RAC for appropriate recognition and follow-up.

6.3 Post-Removal Responsibilities

6.3.1 New York State Department of Environmental Conservation

Through the Rotating integrated Basin Studies (RIBS) program, NYSDEC staff will continue to monitor water quality within Eighteenmile Creek, and staff also will continue to provide management and oversight support for active and inactive contaminated sites within the Eighteenmile Creek watershed.

6.3.2 United States Environmental Protection Agency

The USEPA Great Lakes National Program Office (GLNPO) will continue to provide funding for RAP/RAC Coordination and technical resources support the removal of remaining BUIs and ultimately the delisting of the AOC. USEPA Region 2 will have continued responsibility for addressing the various Eighteenmile Creek Operable Units under the Federal Superfund program.

6.3.3 Niagara County Soil and Water Conservation District

NCSWCD will continue to serve as the RAP coordinator for the Eighteenmile Creek AOC, facilitating RAC meetings, providing technical and administrative assistance for AOC documentation, serving as the primary point of contact for the AOC, and coordinating the overall implementation of the RAP for the Eighteenmile Creek AOC.

6.3.4 Remedial Advisory Committee

The RAC will continue to forward the objectives of the RAP by evaluating, supporting, and documenting the restoration of the Eighteenmile Creek AOC, until all the BUIs are restored and the long-term goal of delisting the AOC can be achieved.

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Appendix 1 – USGS Fish Community Assessment

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Condition of resident fish communities in the Eighteenmile Creek Area of Concern, New York

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ABSTRACT

The lower 3.5 km of Eighteenmile Creek, a tributary to Lake Ontario in New York, was designated as an Area of Concern (AOC) in 1985 under the Great Lakes Water Quality Agreement due to extensive contamination of bed sediments by polychlorinated biphenyls (PCBs) and other toxicants. Five beneficial use impairments (BUIs) have been identified in this AOC, including degraded fish and wildlife populations. We surveyed fish communities in the Eighteenmile Creek AOC and in a comparable section of a nearby reference stream (Oak Orchard Creek) during June 2019 to infer whether legacy contaminants are currently impairing fish communities in the AOC to an extent that they differ from the regional reference condition. Estimates of community abundance, biomass, diversity, and fish condition from each system were compared using a noninferiority testing framework. Biomass, diversity, and fish condition in the Eighteenmile Creek AOC were similar or superior to that in Oak Orchard Creek, while abundance was 20% lower in the AOC. These findings and those of a 2007 sampling effort suggest that fish communities in the Eighteenmile Creek AOC are not impaired despite recent studies indicating that PCBs are bioaccumulating in fish tissues at 1–2 orders of magnitude above background levels. Future assessments in the Eighteenmile Creek AOC might focus on the condition of benthic macroinvertebrate communities and potential toxicity of local contaminants to piscivorous wildlife in order to fully address the remaining aspects of the fish and wildlife populations beneficial use impairment.

Introduction

Eighteenmile Creek, a tributary to Lake Ontario in Niagara County of New York State, was designated as an Area of Concern (AOC) in 1985 under the Great Lakes Water Quality Agreement between the United States and Canada. Areas of Concern are defined as geographic areas impacted by environmental degradation resulting from human activities at the local level and have one or more of 14 possible beneficial use impairments (BUIs) relating to chemical, physical, or biological integrity. This designation was given to Eighteenmile Creek because water quality and bed sediments were contaminated by past industrial and municipal discharges, waste disposal, and pesticide usage (CH2MHILL et al., 2015; NCSWCD, 2011; NYSDEC, 1997; NYSDOH, 2015). In 2012, this AOC and areas upstream of it were also added to the Superfund National Priorities List of the country's most hazardous waste sites (USEPA, 2012). Five beneficial use impairments have been identified in the Eighteenmile Creek AOC, including BUI #3, degraded fish and wildlife populations (NCSWCD, 2011).

The degraded fish and wildlife populations BUI exists for an AOC when "fish and wildlife management programs have identified degraded fish or wildlife populations due to a cause within the watershed" (IJC, 1991). The status of this BUI was originally listed as "Unknown" for the Eighteenmile Creek AOC because empirical biological data were lacking, but strong evidence of pollution was noted in stream habitats (NYSDEC, 1997). More recently, a survey of fish and wildlife populations and fish tissue contaminant concentrations concluded that bird and amphibian populations were not impaired but that fish and mammal populations likely were impaired due to high concentrations of polychlorinated biphenyls (PCBs) measured in brown bullhead *Ameiurus nebulosus* (E&E, 2009). Impairment was not directly observed in fish and mammal populations, however, but rather was inferred due to contaminant levels (NCSWCD, 2011). Research elsewhere has found ample evidence of PCB impacts on mammals; for example, American Mink *Neovison vison* are especially sensitive to dioxin-like co-planar PCBs and can have reproductive failure at

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part-per-billion concentrations in the diet (Brunström et al., 2001; Haynes et al., 2009). A recent review concerning PCB toxicity in fish, however, found little evidence that elevated PCB concentrations directly affect the health or survival of wild fish and the integrity of their populations (Henry, 2015). Thus, much uncertainty remains about whether fish populations are truly impaired in the Eighteenmile Creek AOC or if they are simply expected to be impaired.

The U.S. Geological Survey (USGS) and New York State Department of Environmental Conservation (NYSDEC) initiated the current study during 2019 to gather more extensive information on the condition of fish communities needed to evaluate the fish and wildlife populations BUI in the Eighteenmile Creek AOC. Revised BUI removal criteria adopted in 2019 (NCSWCD, 2019; Pickard et al., 2020) by the Remedial Action Committee for the Eighteenmile Creek AOC state that this BUI can be removed when:

- Fish community metrics (e.g., diversity, abundance, biomass, and condition) are similar to reference site(s); AND
- Benthic macroinvertebrate community composition is within the range expected and similar to reference site condition; AND
- PCB concentrations in fish tissue and other prey are below thresholds likely to result in acute toxicity to fish or piscivorous wildlife (birds and mammals).

The primary objective of this investigation was to evaluate the first removal criterion by determining if fish communities in the Eighteenmile Creek AOC are similar to the regional reference condition where chemical contamination is at background levels. This approach of assessing difference from comparable reference conditions has been used successfully in several other BUI assessments across New York (Baldigo et al., 2012, 2016; Duffy et al., 2016; George et al., 2017) because it helps control for confounding regional stressors such as eutrophication, sedimentation, and invasive species. More important, it is consistent with the International Joint Commission guidelines (IJC, 1991), and a NYSDEC guidance document (NYSDEC, 2010), which describes the goal of the AOC remedial process in New York as ensuring that conditions in an AOC are no worse than those in the surrounding area. The second and third BUI removal criteria are being evaluated by other studies and are beyond the scope of this manuscript.

Study area

The main branch of Eighteenmile Creek is approximately 24 km long and flows north from its headwaters near Lockport to its mouth at Lake Ontario in Olcott, New York. The AOC is defined as the downstream-most section, specifically the 3.5-km reach between a hydroelectric dam (Burt Dam) and Lake Ontario (Fig. 1). Additionally, the entire Eighteenmile Creek watershed has been designated as the source area of the contaminants that degraded the quality of sediments in the AOC because most point sources of sediment contamination were located upstream of the AOC (CH2MHILL et al., 2015; NCSWCD, 2011). Polychlorinated biphenyls, chlorinated pesticides, and heavy metals have been found in bed sediments at concentrations well above NYSDEC standards both within and upstream of the AOC (CH2MHILL et al., 2015; NCSWCD, 2011; NYSDOH, 2015; Pickard, 2006; Stackelberg and Gustavson, 2012). Some upstream source area sediments have significantly higher PCB concentrations than sediments in the AOC and have been measured at concentrations as high as 1,400 mg/kg, exceeding hazardous waste levels (i.e., greater than 50 mg/kg) (CH2MHILL et al., 2015). Within the AOC, PCB sediment concentrations average <0.5 mg/kg and peak values have been measured at <2 mg/kg (CH2MHILL et al., 2015; E&E, 2017; Stackelberg and Gustavson, 2012). In addition to bed sediments, PCBs appear to be mobile in surface waters of the AOC, often at concentrations 1–2 orders of magnitude higher than observed in any other Lake Ontario tributary (USEPA, 2011). In 2017, the U.S. Environmental Protection Agency (USEPA) issued a Record of Decision to remove contaminated sediments within Operable Unit Two (OU2), known as the "creek corridor" site (USEPA, 2017), an approximately 1-mile (1.6 km) section of the source area (upstream of the AOC), while the remaining sections of the creek, including the AOC, are still being investigated. Although remedial actions are pending, sediment remediation had not yet occurred in OU2 or elsewhere in Eighteenmile Creek at the time this study was conducted.

In order to determine if present-day fish communities in the Eighteenmile Creek AOC are representative of regional reference conditions, fish communities were sampled in the AOC and in Oak Orchard Creek, a comparable stream that enters Lake Ontario approximately 43 km to the east (Fig. 1). Oak Orchard Creek is similar in surrounding geography to Eighteenmile Creek and also has a hydroelectric dam (Waterport Dam) near its mouth but it is not known to have extensive point source legacy chemical contamination (E&E, 2009). The downstream reaches of Eighteenmile Creek and Oak Orchard Creek are both drowned river mouth habitat subject to backwater from Lake Ontario and are characterized by cattail beds and little riparian development. The reach of Oak Orchard Creek downstream of Waterport Dam is well established as a reference location for assessments in the Eighteenmile Creek AOC and has been included in prior assessments of the fish tumors and other deformities, fish and wildlife populations, bird or animal deformities or reproductive problems, and benthos BUIs (E&E, 2009; George et al., 2017). This reach has also been selected by the USEPA as a suitable reference area for assessments of Reach 1 of Eighteenmile Creek Superfund Operable Unit 3 (E&E, 2017, 2019). As a result, detailed habitat information was not collected in 2019 as part of this effort and a summary of existing information is presented in Table 1.

Eighteenmile Creek and Oak Orchard Creek are both popular sportfishing destinations because they support annual spawning runs of trout and salmon from Lake Ontario (E&E, 2007; NCSWCD, 2011). These large salmonids and their offspring are only transient members of the fish communities, however, in part because the meandering, low gradient, warm-water habitats characteristic of each creek are unsuitable for cold-water fish much of the year. Therefore, we focused this evaluation on the resident warm-water fish community of each creek, which should be more representative of ambient river conditions.

Methods

Fish community surveys

Fish communities were sampled using daytime boat electrofishing of nearshore habitats on June 10–12, 2019, following methods described in Miranda and Boxrucker (2009) and Moulton et al. (2002). Sampling was conducted using 2 anode booms on a 4.9-m (16-ft) Smith-Root electrofishing boat outfitted with an APEX electrofishing system and EU7000iS Honda 7000-Watt Inverter Generator applying pulsed direct current (voltage: 220 V, frequency: 45 Hz, and duty cycle: 15%). The APEX system records a suite of input and output information at one second intervals during electrofishing. The mean peak current output during sampling on Eighteenmile Creek was 58.7 amps compared to 56.4 amps on Oak Orchard Creek. Three crew members netted stunned fish during sampling. Five subreaches were sampled in both Eighteenmile Creek and in Oak Orchard Creek. Electrofishing was conducted for 1200 s of "on time" at each subreach along parallel near-shore habitats on both stream banks. The subreaches in each creek were

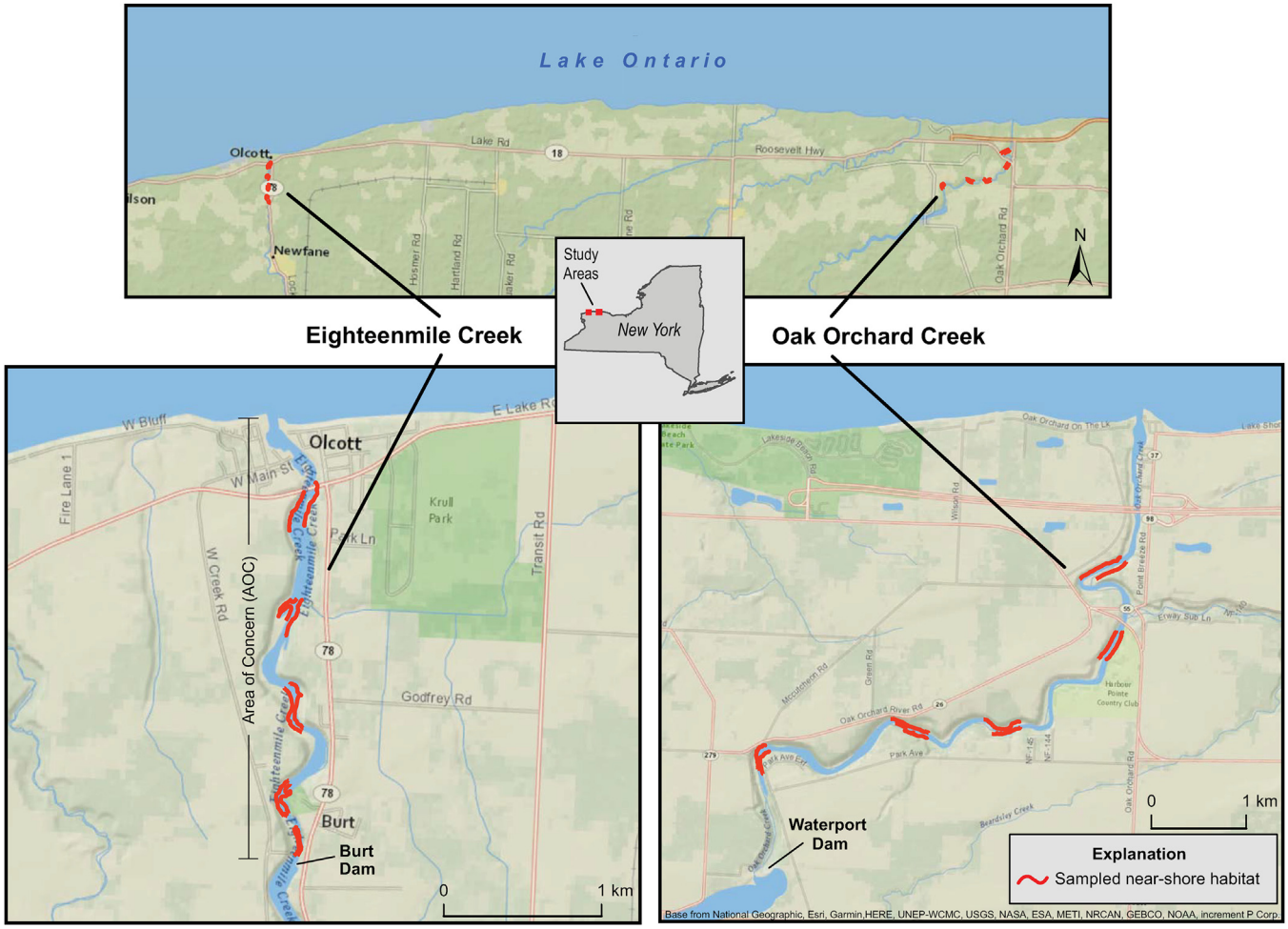

Fig. 1. Location of subreaches in the Eighteenmile Creek AOC and in Oak Orchard Creek where fish communities were sampled.

Table 1
Summary of physical characteristics and chemical parameters from the Eighteenmile Creek AOC and the reach of Oak Orchard Creek downstream of Waterport Dam.

Parameter	Eighteenmile Creek AOC	Oak Orchard Creek (downstream of Waterport Dam)	Period	Data Source
Riparian Corridor Percent Forested (%)	23	26	2002 & 2005	(E&E, 2009)
Riparian Corridor Percent Developed (%)	33	16	2002 & 2005	(E&E, 2009)
Riparian Corridor Percent Agricultural (%)	20	32	2002 & 2005	(E&E, 2009)
Mean annual discharge (m^3/s)	3.98	10.09	2013–2018	USGS streamgages 04219768 and 0422016550[1], respectively (https://waterdata.usgs.gov/nwis)
Watershed area (km^2)	225	704		https://streamstats.usgs.gov/ss/
pH	7.9 (mean, n = 12)	8.1 (mean, n = 8)	2005–2010	(USEPA, 2011)
Specific conductivity (μS/cm)	568 (mean, n = 12)	631 (mean, n = 8)	2005–2010	(USEPA, 2011)
Total suspended solids (mg/L)	4.0 (mean, n = 12)	3.1 (mean, n = 8)	2005–2010	(USEPA, 2011)
Sediment particle size (phi units)	4.8 (mean, n = 3)	4.8 (mean, n = 3)	2014	(George et al., 2017)
Sediment total organic carbon (%)	2.8 (mean, n = 3)	1.7 (mean, n = 3)	2014	(George et al., 2017)
Water mercury concentration (ng/L)	2.8 (mean, n = 4)	0.9 (mean, n = 4)	2009–2010	(USEPA, 2011)
Water PCB concentration (pg/L)	63,500 (mean, n = 4)	275 (mean, n = 4)	2009–2010	(USEPA, 2011)

[1] Streamgage 0422016550 on Oak Orchard Creek is located upstream of Waterport Dam.

distributed relatively evenly between the lake confluence and the upper bounding dam to ensure adequate representation of the entire reach while avoiding areas that were unsafe to sample such as extensive dockage and the immediate tailwaters of each dam (Fig. 1). All fish were identified to species, measured for total length, weighed, and subsequently released.

Data analysis

Statistical analysis was designed to separately evaluate each of the four components of the first BUI removal criterion (diversity, abundance, biomass, and condition). Abundance was assessed by summing the total number of all fish captured in each subreach

and then standardizing by the shocking time to produce an index of relative abundance or catch-per-unit effort (CPUE) as fish per hour. Similarly, biomass was assessed by summing the total mass of all fish captured in each subreach and then standardizing by shocking time to produce an index of relative biomass or grams of fish per hour. Since a uniform 1200 s of sampling was conducted at each subreach, the standardization is not necessary but was performed to provide each index in conventional units and to facilitate comparisons with other studies. Diversity was assessed using two indices: species richness and Shannon's Index of Diversity. Species richness was calculated as the total number of species captured in each subreach and Shannon's Index was calculated as:

$$H' = -\sum_{i=1}^{S}(p_i)(\log_e p_i)$$

where S is the number of species and p_i is the proportion of the total sample represented by the ith species (Kwak and Peterson, 2007).

Fish condition was compared between Eighteenmile Creek and Oak Orchard Creek using relative weight. This analysis was performed for largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, and brown bullhead *Ameiurus nebulosus* because these species were relatively abundant in each creek and span a range of foraging behaviors and trophic positions. Relative weight is a measure of fish plumpness and is used as a surrogate for estimating fish health or condition (Neumann et al., 2012). It is based on the ratio of the observed fish weight to an expected weight based on fish length at capture (Blackwell et al., 2000; Wege and Anderson, 1978). Relative weight was calculated as:

$$Wr = \left(\frac{W}{Ws}\right) \times 100$$

where Wr is the relative weight, W is the weight of a given fish, and Ws is a standard weight for a fish of the observed length across the species geographic range (Neumann et al., 2012; Ogle, 2016; Pope and Kruse, 2007). Standard weights (Ws) were determined from the standard weight equations for largemouth bass (Henson, 1991), bluegill (Hillman, 1982), and brown bullhead (Bister et al., 2000) and were used to calculate Wr for each species with the FSA package (Ogle et al., 2018) in R (R Core Team, 2019). The minimum total length for inclusion in this calculation was 150 mm for largemouth bass, 80 mm for bluegill, and 130 mm for brown bullhead (Ogle et al., 2018). A Wr value of 100 for these species corresponds to the 75th percentile of fish weight for a given length and therefore represents a fish in above-average condition (Neumann et al., 2012). In order to obtain adequate sample size, Wr values for each of the target species were pooled across subreaches at each creek. Linear regression indicated that the relative weight of each species was not significantly related to fish length, so a single mean relative weight was calculated for each species by creek (Pope and Kruse, 2007).

Noninferiority testing was used to compare (a) the abundance, biomass, and diversity indices between the five replicate samples taken from Eighteenmile Creek and Oak Orchard Creek, and (b) the relative weight of largemouth bass, bluegill, and brown bullhead between Eighteenmile Creek and Oak Orchard Creek. This statistical approach flips the traditional hypothesis testing structure and puts the burden of proof on demonstrating equivalence, rather than difference (Lakens, 2017; Mascha and Sessler, 2011; Walker and Nowacki, 2011). Such an approach is appropriate when the goal of management action is to restore the condition of an impacted area to that of the surrounding area and has previously been applied to the BUI-assessment framework (Rutter, 2010). Noninferiority was evaluated using one-sided, two-sample equivalence tests (not assuming equal variance) in Minitab v17. Noninferiority was established only if the entire 95% confidence interval around the ratio of the test mean (AOC) to the reference mean (Oak Orchard Creek) was greater than a lower limit of 0.75 (i.e., establish 95% confidence that the test mean was at least 75% of the reference mean). One of the primary challenges with this type of analysis is determining the degree of departure (tolerance value) from the reference condition that is acceptable. There is little consensus regarding the effect size that monitoring programs should target for detecting change in fish assemblages (see Table 6.8 in Janz et al. (2010)). Therefore in this analysis, 25% was used because it has been recommended as appropriate for many environmental monitoring applications (Munkittrick et al., 2009).

Although a measure of fish community structure is not explicitly identified in the BUI removal criteria, we compared the composition of fish communities between Eighteenmile Creek and Oak Orchard using multivariate techniques to obtain a comprehensive assessment of entire communities. The raw species counts from each subreach were $\log_e(x + 1)$-transformed and used to form a resemblance matrix of Bray-Curtis similarities comparing all 10 subreaches. We then calculated the analysis of similarities (ANOSIM) R-statistic to compare between-group and within-group similarity using PRIMER-E version 7 software (Clarke and Gorley, 2015; Clarke et al., 2014). An R-value of >0.75 indicates well-separated groups, whereas an R-value of 0.50–0.75 indicates separate but abutting or slightly overlapping groups and an R-value of 0.25–0.50 indicates distinguishable but overlapping groups (K. R. Clarke, Plymouth Marine Laboratory, personal communication; Ramette, 2007). The similarity percentages (SIMPER) technique was then used to identify the species that contributed most strongly to differences between the creeks (Clarke and Gorley, 2015).

Results

The electrofishing surveys collected a total of 487 individuals from 33 species in Eighteenmile Creek and 612 individuals from 34 species in Oak Orchard Creek (Table 2). The raw fish survey data are available in a USGS data release (George, 2020). The most frequently captured species in Eighteenmile Creek was smallmouth bass *Micropterus dolomieu* while the most frequently captured species in Oak Orchard Creek was golden redhorse *Moxostoma erythrurum*. Most species were present in both creeks, with a total of 29 common species. Black redhorse *Moxostoma duquesnei* (n = 1), goldfish *Carassius auratus* (n = 1), and two sunfish hybrids (n = 1) were unique to Eighteenmile Creek while black crappie *Pomoxis nigromaculatus* (n = 1), fathead minnow *Pimephales promelas* (n = 1), freshwater drum *Aplodinotus grunniens* (n = 8), spotted gar *Lepisosteus oculatus* (n = 1), and walleye *Sander vitreus* (n = 3) were only captured in Oak Orchard Creek (Table 2). The spotted gar from Oak Orchard Creek, although not a focus of this investigation, is the only confirmed specimen of this species captured in New York waters (https://nas.er.usgs.gov/viewer/omap.aspx?SpeciesID=756, accessed Feb. 2, 2020). It is unclear at this time if this capture represents an established population, aquarium release, or other phenomena.

Total community relative abundance (or CPUE) ranged from 147 to 546 fish/h across the five subreaches in Eighteenmile Creek and from 156 to 573 fish/h at Oak Orchard Creek. The mean CPUE was 292 fish/h at Eighteenmile Creek compared to 367 fish/h at Oak Orchard Creek. Noninferiority of Eighteenmile Creek was not established for this metric (T = 0.196, P = 0.425) as the lower bound of the 95% confidence interval about the ratio of the Eighteemile Creek and Oak Orchard Creek means extended to 0.41 (below the threshold value of 0.75) (Table 3).

Relative biomass ranged from 139,937–326,382 g/h across the five subreaches in Eighteenmile Creek and from 88,246–319,733 g/h at Oak Orchard Creek. The mean relative biomass

Table 2
Number and combined mass of all fish captured by species across five subreaches on Eighteenmile Creek and Oak Orchard Creek sampled June 2019. A "–" indicates no fish were captured.

Common Name	Scientific Name	Number of fish captured		Biomass of fish captured (g)	
		Eighteenmile Creek	Oak Orchard Creek	Eighteenmile Creek	Oak Orchard Creek
American Eel	*Anguilla rostrata*	1	1	2088	637
Black Crappie	*Pomoxis nigromaculatus*	–	1	–	245
Black Redhorse	*Moxostoma duquesnei*	1	–	892	–
Bluegill	*Lepomis macrochirus*	34	47	4245	5348
Bluntnose Minnow	*Pimephales notatus*	7	6	29	14
Bowfin	*Amia calva*	7	4	13,815	8433
Brown Bullhead	*Ameiurus nebulosus*	17	27	8138	11,530
Common Carp	*Cyprinus carpio*	31	12	183,240	57,973
Common Shiner	*Luxilus cornutus*	5	2	24	8
Emerald Shiner	*Notropis atherinoides*	10	8	28	23
Fathead Minnow	*Pimephales promelas*	–	1	–	1
Freshwater Drum	*Aplodinotus grunniens*	–	8	–	25,355
Gizzard Shad	*Dorosoma cepedianum*	9	9	8111	7069
Golden Redhorse	*Moxostoma erythrurum*	41	231	34,036	125,193
Golden Shiner	*Notemigonus crysoleucas*	8	2	63	9
Goldfish	*Carassius auratus*	1	–	1291	–
Greater Redhorse	*Moxostoma valenciennesi*	3	23	6784	23,035
Green Sunfish X Bluegill	*L. cyanellus X L. macrochirus*	1	–	160	–
Largemouth Bass	*Micropterus salmoides*	36	22	23,697	11,024
Logperch	*Percina caprodes*	5	3	38	18
Longnose Gar	*Lepisosteus osseus*	3	6	1569	4748
Mimic Shiner	*Notropis volucellus*	7	5	15	9
Northern Pike	*Esox lucius*	1	3	1524	6494
Pumpkinseed	*Lepomis gibbosus*	26	37	2169	2837
Pumpkinseed X Bluegill	*L. gibbosus X L. macrochirus*	1	–	89	–
Rock Bass	*Ambloplites rupestris*	47	31	9248	4111
Rudd	*Scardinius erythrophthalmus*	10	1	6657	1261
Shorthead Redhorse	*Moxostoma macrolepidotum*	14	7	8792	4212
Silver Redhorse	*Moxostoma anisurum*	2	4	3717	9844
Smallmouth Bass	*Micropterus dolomieu*	81	17	73,946	10,579
Spotfin Shiner	*Cyprinella spiloptera*	1	1	8	4
Spottail Shiner	*Notropis hudsonius*	2	1	14	9
Spotted Gar	*Lepisosteus oculatus*	–	1	–	503
Striped Shiner	*Luxilus chrysocephalus*	43	54	380	266
Walleye	*Sander vitreus*	–	3	–	5510
White Perch	*Morone americana*	1	7	18	1084
White Sucker	*Catostomus commersonii*	24	5	15,802	2714
Yellow Perch	*Perca flavescens*	7	22	1134	1562
Total		**487**	**612**	**411,761**	**331,659**

was 247,057 g/h at Eighteenmile Creek compared to 198,996 g/h at Oak Orchard Creek. Noninferiority of Eighteenmile Creek was established for this metric (T = 2.129, P = 0.035) (Table 3).

Richness ranged from 13 to 21 species across the five subreaches in Eighteenmile Creek and from 14 to 22 species at Oak Orchard Creek. The mean richness was 18.0 at Eighteenmile Creek compared to 18.6 at Oak Orchard Creek. Noninferiority of Eighteenmile Creek was established for this metric (T = 2.377, P = 0.025) (Table 3). Shannon's Index ranged from 2.17 to 2.76 across the five subreaches in Eighteenmile Creek and from 2.07 to 2.43 at Oak Orchard Creek. The mean value was 2.47 at Eighteenmile Creek compared to 2.18 at Oak Orchard Creek. Noninferiority of Eighteenmile Creek was established for this metric (T = 6.821, P = <0.001) (Table 3).

The condition of all three species assessed in Eighteenmile Creek was similar to or greater than that of Oak Orchard Creek. A total of 36 largemouth bass were captured on Eighteenmile Creek ranging in length from 103 to 486 mm compared to 22 on Oak Orchard Creek ranging in length from 164 to 402 mm. All but three individuals from Eighteenmile Creek met the minimum length requirement of 150 mm for inclusion in the calculation of relative weight. The mean relative weight of largemouth bass was 105.9 at

Table 3
The mean, standard error (SE), and results of noninferiority tests comparing relative abundance, relative biomass, species richness, and Shannon's Index between five replicate subreaches in each creek and the relative weight (*Wr*) of largemouth bass, bluegill, and brown bullhead between creeks. P-values ≤0.05 are bolded and indicate that noninferiority was established for Eighteenmile Creek.

	Eighteenmile Creek mean (SE)	Oak Orchard Creek mean (SE)	Noninferiority Test Results		
			T-value	P-value	df
Relative Abundance (fish/h)	292 (69)	367 (68)	0.196	0.425	7
Relative Biomass (g/h)	247,057 (34,078)	198,996 (41,079)	2.129	**0.035**	7
Richness (no. species)	18.0 (1.3)	18.6 (1.4)	2.377	**0.025**	7
Shannon's Index	2.47 (0.11)	2.18 (0.06)	6.821	**<0.001**	6
Wr Largemouth Bass	105.9 (1.8)	100.4 (1.4)	14.805	**<0.001**	52
Wr Bluegill	113.8 (2.0)	104.5 (1.3)	16.193	**<0.001**	58
Wr Brown Bullhead	93.2 (1.8)	87.7 (2.3)	11.233	**<0.001**	41

Eighteenmile Creek compared to 100.4 at Oak Orchard Creek and noninferiority of Eighteenmile Creek was established for this species (T = 14.805, P < 0.001) (Table 3). A total of 34 bluegill were captured on Eighteenmile Creek ranging in length from 100 to 195 mm compared to 47 on Oak Orchard Creek ranging in length from 63 to 194 mm. All but one individual from Oak Orchard Creek met the minimum length requirement of 80 mm for inclusion in the calculation of relative weight. The mean relative weight of bluegill was 113.8 at Eighteenmile Creek compared to 104.5 at Oak Orchard Creek and noninferiority of Eighteenmile Creek was established for this species (T = 16.193, P < 0.001) (Table 3). A total of 17 brown bullhead were captured on Eighteenmile Creek ranging in length from 252 to 393 compared to 27 on Oak Orchard Creek ranging in length from 250 to 388, and all individuals met the minimum length requirement of 130 mm for inclusion in the calculation of relative weight. The mean relative weight of brown bullhead was 93.2 at Eighteenmile Creek compared to 87.7 at Oak Orchard Creek, and noninferiority of Eighteenmile Creek was established for this species as well (T = 11.233, P < 0.001) (Table 3).

The composition of fish communities at Eighteenmile Creek was similar to that of Oak Orchard Creek. Twenty-nine species were common to both creeks, while only 4 species were unique to Eighteenmile Creek and 5 species were unique to Oak Orchard Creek (Table 2). The mean Bray-Curtis similarity within the five subreaches from Eighteenmile Creek was 56.2 compared to 58.2 within the five subreaches at Oak Orchard Creek, while the mean similarity between the subreaches from each creek was 54.6. The ANOSIM *R*-statistic comparing ranked between- and within-group similarity was $R = 0.28$, suggesting barely distinguishable grouping between the samples from each creek. The SIMPER analysis indicated that differences in the abundance and presence of golden redhorse, striped shiner *Luxilus chrysocephalus*, greater redhorse *Moxostoma valenciennesi*, and smallmouth bass were responsible for the most dissimilarity between the five subreaches of each creek. The relative abundance of smallmouth bass was greater at Eighteenmile Creek, while the relative abundances of golden redhorse, striped shiner, and greater redhorse were greater at Oak Orchard Creek. Full results from the SIMPER analysis are available in Table S1. Of the 29 species common to both creeks, 18 were equally represented or more abundant at Eighteenmile Creek (Table 2).

Discussion

The primary purpose of this investigation was to determine if fish communities in the Eighteenmile Creek AOC are similar to the regional reference condition where chemical contamination is at background levels. Our results indicate that indices of biomass, diversity, and fish condition in the Eighteenmile Creek AOC were similar or superior to those of the comparable reference, Oak Orchard Creek. Mean relative abundance was 20% lower in Eighteenmile Creek, and the collected data did not meet the *a priori*-defined criteria for noninferiority. The difference in relative abundance was driven almost exclusively by a high density of redhorse suckers (*Moxostoma* sp.) in Oak Orchard Creek. Although redhorse suckers are often considered to be sensitive to pollution and habitat degradation (Grabarkiewicz and Davis, 2008), individuals of this genus were also present in all five subreaches on Eighteenmile Creek so it is unclear if the observed difference is meaningful. Mean relative biomass was 24% greater in Eighteenmile Creek than in Oak Orchard Creek, which clearly indicated that this metric was not inferior in the AOC. Of the two diversity metrics utilized, species richness was nearly equivalent between the two creeks while Shannon's Index was 13% greater in Eighteenmile Creek. The mean condition or relative weight (*Wr*) of the three representative species, largemouth bass, bluegill, and brown bullhead, indicated that fish condition in the AOC compared favorably to that observed in Oak Orchard Creek. Furthermore, the mean *Wr* of largemouth bass and bluegill in Eighteenmile Creek was greater than 100, suggesting these species exceeded the 75th percentile and therefore were in above average condition (Neumann et al., 2012). Finally, a multivariate analysis of count data for all species populations suggested that the composition of fish communities was relatively similar between Eighteenmile Creek and Oak Orchard Creek although some grouping by creek was evident.

The results of this investigation complement findings from a similar study conducted in 2007 (E&E, 2009), and together provide a comprehensive picture of fish communities in the Eighteenmile Creek AOC. The prior study assessed fish communities in the Eighteenmile Creek AOC and Oak Orchard Creek during May and August 2007 and concluded that fish communities were relatively similar between the two creeks. During the May 2007 survey, mean relative abundance (CPUE) was 408 fish/h in Eighteenmile Creek and 288 fish/h in Oak Orchard Creek, while comparable values during the August 2007 survey were 210 fish/h and 78 fish/h for Eighteenmile Creek and Oak Orchard Creek, respectively (E&E, 2009). Similarly, during the May 2007 survey, 23 species were captured in Eighteenmile Creek compared to 21 in Oak Orchard Creek, and comparable values during the August 2007 sampling were 16 and 14 species, respectively (E&E, 2009). It is unclear if the differences in relative abundance and richness between the 2007 and the 2019 surveys are attributable to sampling efficiency, seasonal differences in communities, changes to communities in the 12 years between surveys, or other factors. However, the results from 2007 and 2019 largely indicate that the condition of fish communities in the Eighteenmile Creek AOC is similar or superior to the reference condition. Together, the combined results from both studies provide a more robust assessment by comparing fish communities between Eighteenmile Creek and Oak Orchard Creek during three independent surveys conducted in different months: May and August 2007 (E&E, 2009) and June 2019 in the present investigation.

It is not completely unexpected that the health of individual fish and their communities in the Eighteenmile Creek AOC are in good condition, despite the presence of contaminated sediments and evidence that PCBs are bioaccumulating in resident fish. The mean whole-body PCB concentration in brown bullhead in 2007 was an order of magnitude higher in Eighteenmile Creek (3.2 mg/kg) than in Oak Orchard Creek (0.187 mg/kg) (E&E, 2009), while composited forage fish samples from 2018 averaged 2.1 mg/kg in the AOC compared to 0.079 mg/kg in Oak Orchard Creek (E&E, 2019). The absence of detectable community or population-level effects in spite of elevated tissue concentrations is supported by a recent review of the ecotoxicology of PCBs in fish which concluded there is little evidence that PCBs have any widespread effect on the health or survival of wild fish (Henry, 2015). Similarly, a recent assessment in the St. Clair River AOC (Ontario, Canada) did not find evidence of fish tissue PCB concentrations affecting fish health (Muttray et al., 2020). Several other reviews, however, found stronger evidence for more direct negative effects of elevated tissue PCB concentrations on fish survival, growth, and reproduction (Berninger and Tillitt, 2019; Monosson, 2000), although few of the species encountered in our study were evaluated. The quality of physical habitat in Eighteenmile Creek, though not directly assessed, may explain why adverse effects of contaminated sediments were not more evident. An investigation of the cumulative effects of multiple contaminants on fish communities across Ohio concluded that habitat quality was the single best predictor of fish-community condition, and more important than body burdens of organic chemicals or metals (Dyer et al., 2000). This finding may be relevant to the Eighteenmile Creek AOC where it appears that overall habitat quality is sufficiently high to support fish communities that meet or exceed the regional reference condition.

Although a detailed habitat assessment or deeper investigation as to why fish communities in the AOC are not adversely affected is beyond the scope of this BUI assessment, it is plausible that any potential adverse effects to fish communities from PCB-toxicity are masked by habitat quality or other uncontrolled factors such as immigration from Lake Ontario populations.

The results of this investigation and those of the 2007 survey (E&E, 2009) provide fairly consistent evidence that the condition of fish communities in the Eighteenmile Creek AOC is similar or superior to the condition of fish communities in Oak Orchard Creek and likely other comparable systems across the region. Although noninferiority was not established for fish abundance in our analysis, this finding may reflect variability between subreaches or limited sample size. Surveys conducted during two seasons in 2007 found no evidence of impairment to abundance in the Eighteenmile Creek AOC (E&E, 2009). Thus, the weight of evidence from both studies suggests the first removal criterion of the fish and wildlife populations BUI has been met and future efforts should focus on evaluating aspects of the second and third removal criteria. The second criterion concerns the condition of benthic macroinvertebrate communities and the third criterion concerns PCB concentrations in prey fish and other aquatic or riparian taxa. These two criteria serve largely as surrogates for measuring populations of piscivorous wildlife (i.e., birds and mammals), which have been difficult to consistently document given the small size of the AOC. The current information on the status of benthic macroinvertebrate communities is somewhat inconclusive (E&E, 2013; George et al., 2017), while an investigation is underway to determine the impairment status of mink (a piscivorous mammal) by modeling the potential impacts of PCB-laden fish and other aquatic prey through dietary exposure. Thus, collecting additional data on the condition of benthic macroinvertebrates and obtaining the results of the mink prey study should be prioritized in the near future. Together, that information combined with the fish community information summarized here, should provide a comprehensive suite of data that can be used to determine if the degraded fish and wildlife populations BUI is still warranted in the Eighteenmile Creek AOC.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2020.10.003.

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Appendix 2 – USGS Benthos Assessments

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Comprehensive assessment of macroinvertebrate community condition and sediment toxicity in the Eighteenmile Creek Area of Concern, New York, 2021

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ABSTRACT

The degradation of benthic communities (benthos) is one of four remaining beneficial use impairments (BUIs) in the Eighteenmile Creek Area of Concern (AOC), located on the south shore of Lake Ontario in New York. The historical rationale for listing this BUI as impaired relied heavily on inferred or expected impact to benthic communities based on elevated contaminant concentrations in bed sediments from past industrial and municipal discharges, hazardous-waste disposal, and pesticide usage. Previous assessments of macroinvertebrate community condition in the AOC have produced inconclusive results, and it remains unclear if contaminated sediments are impairing benthic communities. In 2021, a comprehensive assessment of macroinvertebrate community condition and sediment toxicity was conducted at eight sites in the AOC and six sites in a reference area on Oak Orchard Creek to determine if the removal criteria for this BUI have been met or if additional remedial measures are needed. The New York multi-metric index of biological integrity classified the mean community condition across AOC sites as slightly impacted, and 10-day toxicity tests with *Chironomus dilutus* and *Hyalella azteca* found no evidence of toxicity in AOC sediments. Equivalence testing indicated that community condition, and survival and growth of both test species, were not inferior in the AOC relative to the reference area. The weight of evidence from this study and other relevant datasets indicate that sediment contamination is not causing measurable impairment to benthic communities in the Eighteenmile Creek AOC.

1. Introduction

Eighteenmile Creek, a tributary to Lake Ontario in Niagara County of New York State, was designated as an Area of Concern (AOC) in 1985 under the Great Lakes Water Quality Agreement between the United States and Canada. Areas of Concern are defined as geographic areas impacted by environmental degradation resulting from human activities at the local level and have one or more of 14 possible beneficial use impairments (BUIs) relating to chemical, physical, or biological integrity. Eighteenmile Creek received this designation because water quality and bed sediments were contaminated by past industrial and municipal discharges, hazardous-waste disposal, and pesticide usage (CH2MHILL et al., 2015; NCSWCD, 2011; NYSDEC, 1997; NYSDOH, 2015). In 2012, the AOC and areas upstream of it were also added to the Superfund National Priorities List of the country's most hazardous waste sites (USEPA, 2012). Five beneficial use impairments were originally identified in the Eighteenmile Creek AOC, including BUI #6, "degradation of benthos" (NCSWCD, 2011).

The degradation of benthos BUI exists for an AOC when "*benthic macroinvertebrate community structure significantly diverges from unimpacted control sites of comparable physical and chemical characteristics*" or "*toxicity…of sediment associated contaminants at a site is significantly higher than controls*" (IJC, 1991). The status of this BUI was listed as impaired in the Eighteenmile Creek AOC as a result of assessments conducted between 1977 and 1994, which suggested macroinvertebrate communities were adversely affected by contaminated surficial sediments (NYSDEC, 1997; NCSWCD, 2011). These assessments, however, relied heavily on inferred or expected impact to benthic communities based on elevated contaminant concentrations in bed sediments. The limited direct sampling of benthic communities indicated moderate or slight impairment based on community indices, and sediment toxicity tests only suggested evidence of toxicity in one end point for one test species (Abele et al.,

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1998; NYSDEC, 1997). More recent assessments of benthic community condition have also produced inconclusive or inconsistent results. A study conducted in 2012 at five sites in the AOC found that community condition ranged from moderately impacted to slightly impacted, and sediment toxicity tests indicated no evidence of toxicity (E&E, 2013). This study did not include a comparison to a reference area, however, which is necessary to meet the most recent BUI removal criteria. A separate study conducted in 2014 that included three sites in the AOC found community condition ranged from severely impacted to non-impacted and possible evidence of sediment toxicity was observed at one site (George et al., 2017). This study included a comparison to a reference area on Oak Orchard Creek which indicated the condition of benthic communities and the toxicity of sediments were similar between the reference area and the AOC. In 2019, chronic sediment toxicity testing was conducted at three sites in the AOC, as well as upstream source areas and a reference area on Oak Orchard Creek as part of the Remedial Investigation for the Superfund Program. The test endpoints indicated little or no chronic toxicity associated with AOC sediments when compared to the reference site or control sediment samples, although sediments in the source areas of Eighteenmile Creek upstream of the AOC were toxic to test species (WSP, 2021, 2022). Together, the limited amount of community information, age of the data, and inconclusive nature of the findings from these studies has confounded efforts by the Remedial Advisory Committee for the Eighteenmile Creek AOC to determine the status of the benthos BUI. Thus, a more comprehensive investigation was planned to obtain a robust suite of data on the condition of benthic communities to conclusively determine if the removal criteria for the benthos BUI have been met.

The U.S. Geological Survey (USGS) and New York State Department of Environmental Conservation (NYSDEC) initiated the current study during 2021 to address this uncertainty and gather a comprehensive suite of information on the condition of benthic macroinvertebrate communities needed to fully evaluate the status of the Degradation of Benthos BUI in the Eighteenmile Creek AOC. Revised BUI removal criteria adopted in 2020 (NCSWCD, 2020; Pickard et al., 2020) by the Remedial Advisory Committee for the Eighteenmile Creek AOC state that this BUI can be removed when:

-Benthic macroinvertebrate communities are “non-impacted” or “slightly impacted” according to NYSDEC indices; OR

-Benthic macroinvertebrate community condition is similar to unimpacted control sites of comparable physical and chemical characteristics; AND

-Toxicity of sediment-associated contaminants is similar to unimpacted control sites of comparable physical and chemical characteristics

The primary objective of this investigation was to assess benthic community condition to determine if (a) the first removal criterion has been achieved, or (b) both the second *and* third removal criteria have been achieved. This effort involved sampling macroinvertebrate communities to characterize community condition and collecting bed sediments to assess toxicity at sites in the AOC as well as at reference sites located outside of the AOC where chemical contamination is at background levels. Data from nearby reference area(s) are crucial to BUI assessments because they provide a benchmark for gauging the status of any given BUI in the AOC relative to conditions across the region. Grapentine (2009) defined reference as “the conditions representative of the natural, background, or hypothetically expected state of a descriptor of benthic conditions in the absence of the stressor(s) of concern”. This approach of assessing difference from comparable reference conditions has been used successfully in numerous other BUI assessments across New York (Baldigo et al., 2012; Baldigo et al., 2016; Duffy et al., 2016; Duffy et al., 2017; George et al., 2022b) and elsewhere (Scudder Eikenberry et al., 2019; Stevack et al., 2020) because it helps control for confounding regional stressors such as eutrophication, hydrologic modification, and invasive species. It is also consistent with the International Joint Commission guidelines (IJC, 1991), and a NYSDEC guidance document (NYSDEC, 2010), which describes the goal of the AOC remedial process in New York as ensuring that conditions in an AOC are no worse than those in the surrounding area.

2. Methods

2.1. Study area

Predominant land use in the Eighteenmile Creek watershed consists of agriculture, forest, and developed land, and the vast majority of high-intensity land use takes place near the City of Lockport. The main branch of Eighteenmile Creek is approximately 24 km long and flows north from its headwaters near Lockport to its mouth at Lake Ontario in Olcott, New York. The AOC is defined as the downstream-most section, specifically the 3.5-km reach between a hydroelectric dam (Burt Dam) and Lake Ontario (Fig. 1). Stream habitats in the AOC range from approximately 30–90 m in width and 0.5–3.5 m in depth (E&E, 2003) and the annual mean discharge below Burt Dam averaged 3.95 m^3/s between 2012 and 2021 (U.S. Geological Survey, 2022).

The entire Eighteenmile Creek watershed has been designated as the source area of the contaminants that degraded the quality of sediments in the AOC because most point sources of sediment contamination were located upstream of the AOC (CH2MHILL et al., 2015; NCSWCD, 2011). As a result, chemical contamination of the sediment in Eighteenmile Creek generally increases in concentration moving from downstream (AOC) to upstream (source) areas. Polychlorinated biphenyls (PCBs), chlorinated pesticides, and heavy metals have been found in bed sediments at concentrations well above NYSDEC standards both within and upstream of the AOC (CH2MHILL et al., 2015; NCSWCD, 2011; NYSDOH, 2015; Pickard, 2006; Stackelberg and Gustavson, 2012). PCB and lead concentrations follow a similar spatial distribution in creek sediments, increasing from downstream to upstream, but a greater percentage of lead samples (85% and 35%, respectively) exceed the NYSDEC Class C screening criteria (NYSDEC, 2014), indicating these sediments are likely to pose a risk to aquatic life. Additionally, a paired evaluation of metal concentrations and toxicity suggest that metals in sediment may be a causative agent of toxicity to benthic invertebrates in the upper reaches of Eighteenmile Creek (WSP, 2022). In 2017, the U.S. Environmental Protection Agency (USEPA) issued a Record of Decision to remove contaminated sediments within Operable Unit Two (USEPA, 2017), an approximately 1.6 km section of the source area (upstream of the AOC). The USEPA subsequently completed the Remedial Investigation of the remaining sections of the creek (WSP, 2022), including the AOC, but sediment remediation had not yet occurred in any Eighteenmile Creek operable unit at the time this study was conducted.

Sediment samples were collected from eight sites in the Eighteenmile Creek AOC and at six sites in a reference area on Oak Orchard Creek, a comparable stream that enters Lake Ontario approximately 43 km to the east (Fig. 1, Table 1). Oak Orchard Creek is similar in surrounding geography to Eighteenmile Creek and also has a hydroelectric dam (Waterport Dam) near its mouth but is not known to have extensive point source legacy chemical contamination (WSP, 2022; E&E, 2009). The downstream reaches of Eighteenmile Creek and Oak Orchard Creek are both drowned river mouth habitat subject to backwater from Lake Ontario and are characterized by cattail beds and little riparian development. The reach of Oak Orchard Creek downstream of Waterport Dam is well established as a reference location for assessments in the Eighteenmile Creek AOC and has been included in prior assessments of the fish tumors and other deformities, fish and wildlife populations, bird or animal deformities or reproductive problems, and benthos BUIs (E&E, 2009; George et al., 2022a; George et al., 2017). This reach has also been selected by the USEPA as a suitable reference area for assessments of Eighteenmile Creek Superfund Operable Unit 3 (E&E, 2017, E&E, 2019). A more detailed comparison of the habitat and watershed characteristics of both streams is available in Table 1 of George et al. (2022a). Of the eight sites in the AOC, five were randomly selected from a gridded map using a random number generator and the remaining three repeated the

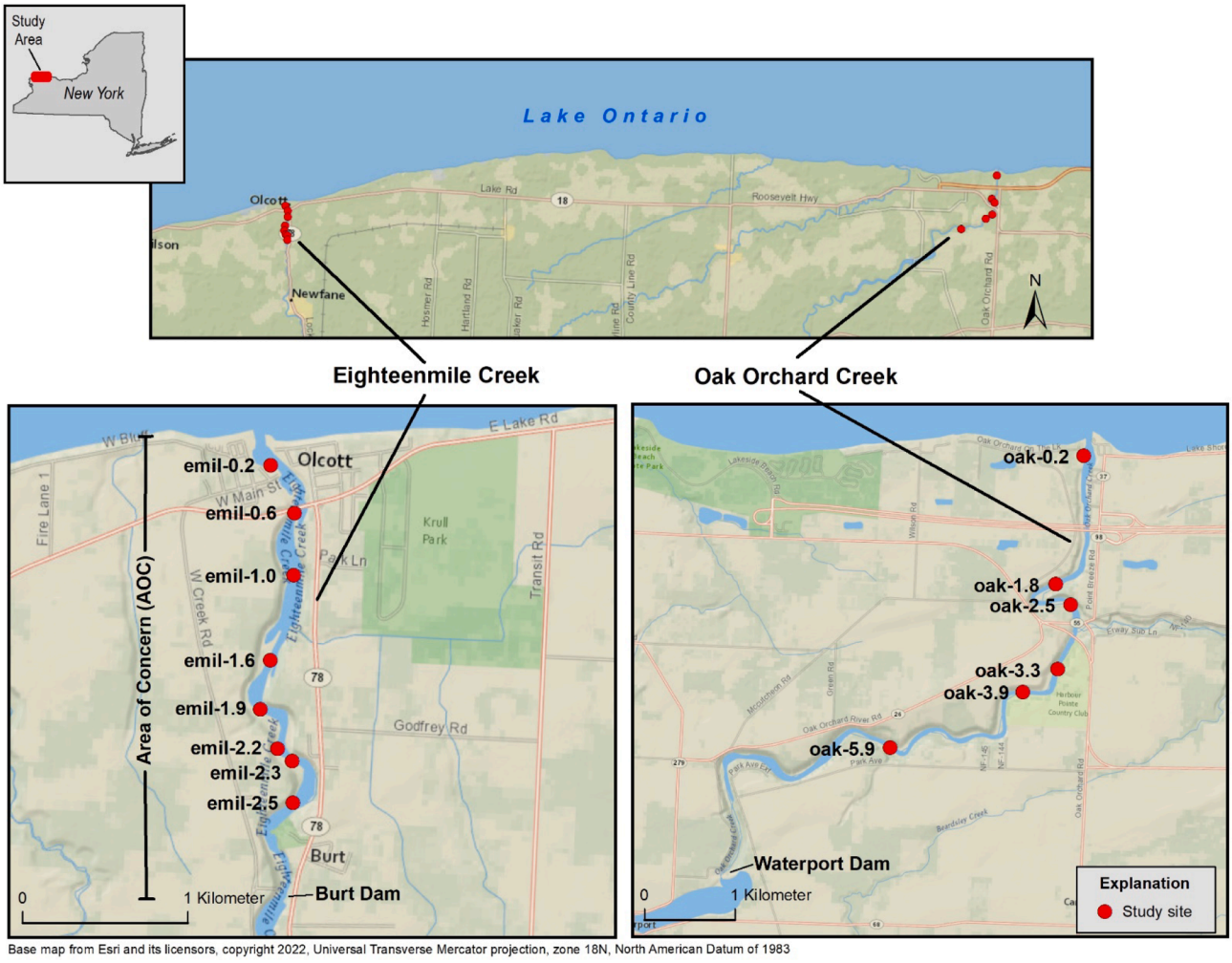

Fig. 1. Map of the study area showing eight sites in the Eighteenmile Creek AOC and six reference sites on Oak Orchard Creek where bed sediments were sampled in 2021.

Table 1
Site information and habitat measurements for eight sites in the Eighteenmile Creek AOC and six sites in the Oak Orchard Creek reference area where sediment samples were collected between August 3–5, 2021. Site IDs are an alphanumeric code that include an approximate measure of river kilometers upstream from Lake Ontario. Data from George and Baldigo (2022).

Water Body	Site ID	Type	Latitude	Longitude	Depth (m)	Temperature (°C)	Specific Conductance (µS/cm)	pH	Dissolved Oxygen (mg/L)	Fine Sediment (%)	Total Organic Carbon (%)
Eighteenmile Creek	emil-0.2	AOC	43.33755	−78.71780	2.2	22.0	757	8.40	8.51	73.0	2.3
Eighteenmile Creek	emil-0.6	AOC	43.33501	−78.71586	4.0	21.0	756	7.91	6.51	76.2	5.1
Eighteenmile Creek	emil-1.0	AOC	43.33162	−78.71571	3.5	20.9	756	8.02	5.90	75.5	6.0
Eighteenmile Creek	emil-1.6	AOC	43.32692	−78.71717	3.4	21.1	673	8.20	6.16	65.2	4.5
Eighteenmile Creek	emil-1.9	AOC	43.32423	−78.71775	1.9	22.1	703	8.74	5.52	31.2	2.7
Eighteenmile Creek	emil-2.2	AOC	43.32212	−78.71635	2.0	21.8	768	8.80	4.34	39.6	5.6
Eighteenmile Creek	emil-2.3	AOC	43.32148	−78.71521	2.0	23.0	768	9.06	7.98	42.5	8.8
Eighteenmile Creek	emil-2.5	AOC	43.31921	−78.71502	1.2	24.2	758	9.16	10.20	39.5	4.3
Oak Orchard Creek	oak-0.2	Reference	43.36998	−78.19265	1.9	23.0	648	8.00	6.30	60.0	4.4
Oak Orchard Creek	oak-1.8	Reference	43.35710	−78.19576	2.7	23.7	647	8.27	7.81	41.6	3.9
Oak Orchard Creek	oak-2.5	Reference	43.35510	−78.19363	1.8	23.6	647	8.16	7.43	38.0	3.5
Oak Orchard Creek	oak-3.3	Reference	43.34867	−78.19507	3.2	22.8	647	7.80	5.05	37.6	1.5
Oak Orchard Creek	oak-3.9	Reference	43.34625	−78.19968	3.8	23.3	660	7.89	5.60	60.3	3.5
Oak Orchard Creek	oak-5.9	Reference	43.34023	−78.21744	1.7	23.1	660	7.82	6.90	45.6	2.6

AOC sites sampled in the 2014 study (George et al., 2017). Similarly, of the six reference sites on Oak Orchard Creek, three were randomly selected and the other three repeated the reference sites sampled in 2014.

2.2. Sample collection and processing

Bed sediments were collected from depositional areas at each site using a petite Ponar (0.03 m^2) dredge on August 3–5, 2021 for use in macroinvertebrate community assessment, sediment toxicity tests, and

assessment of habitat comparability. For macroinvertebrate identification, three replicate samples were collected from each site. A large quantity of sediment was processed for macroinvertebrate samples because past benthic community assessments of non-wadeable habitats in the AOC have struggled to reach the 100-organism target for calculating NYSDEC indices (E&E, 2013; George et al., 2017). Each replicate was composed of the detritus from eight composited grabs that were sieved through a 500 μm mesh screen bottom bucket. The volume of detritus retained by the sieve from the eight composited grabs typically ranged from 1 to 4 L. All detritus was retained for each replicate, preserved with 95% ethanol, and shipped to Watershed Assessment Associates (Schenectady, NY) for identification. For sediment toxicity tests, six grabs were collected from each site, composited and mixed in a bucket, and a 4-L subsample was stored in a polyethylene container. Samples were kept on ice and shipped to Great Lakes Environmental Center, Inc. (Traverse City, MI) where testing was initiated within five weeks of sample collection. For habitat characterization, sediment subsamples were collected from the unused toxicity composite and shipped to RTI Laboratories (Livonia, MI) for measurement of grain-size distribution and total organic carbon (TOC) concentration. Sediment contaminant concentrations (PCBs, metals, etc.) were not analyzed as part of this project because other recent studies had already provided an extensive assessment (WSP, 2022).

Macroinvertebrates were identified following NYSDEC Standard Operating Procedures (Duffy, 2021). A 100-organism subsample, or an exhaustive pick when < 100 organisms were present, was randomly sorted from each macroinvertebrate community replicate using a gridded tray and identified to the lowest practical taxonomic resolution (usually genus). The NYSDEC multi-metric index of biological integrity for Ponar samples was then calculated to assess the condition of macroinvertebrate communities (Duffy, 2021). The index calculates five component metrics: species richness, Hilsenhoff Biotic Index (Hilsenhoff, 1987), Dominant-3, Percent Model Affinity (Novak and Bode, 1992), and the Shannon Diversity Index, and converts them to a standardized value on a scale from 0 to 10. The five component metrics are then averaged to produce the Biological Assessment Profile (BAP) score, a single value for which a four-tiered scale of water quality impact (severe: 0.0–2.5; moderate: 2.5–5.0; slight: 5.0–7.5; or non-impacted: 7.5–10.0) has been established (Duffy, 2021). Impact categories of moderate and severe are considered indicative of impaired conditions (Duffy, 2021). The BAP score (and associated impact tiers) is used to assess water quality and ecosystem condition in surface waters across New York and has been used as the primary metric for assessing benthic condition in the six AOCs in the state (Baldigo et al., 2023; Duffy et al., 2016; Duffy et al., 2017; George et al., 2022b).

Samples for sediment toxicity tests were used to quantify acute and sublethal toxicity to the dipteran, *Chironomus dilutus*, and the amphipod, *Hyalella azteca*, during 10-day survival and growth bioassays following USEPA test methods 100.2 and 100.1, respectively (USEPA, 2000). *Chironomus dilutus* and *H. azteca* are used as indicator species because they each inhabit broad geographic ranges, burrow in sediments, and have known sensitivities to common nutrients and toxins (ASTM, 2010; USEPA and USACE, 1998b; USEPA, 2000). Porewater testing for ammonia (total ammonia as N) was conducted on all samples upon receipt at the testing facility to determine if mitigation was necessary to reduce ammonia concentrations below the 20 mg/L threshold for test initiation following standard procedures (USEPA and USACE, 1998a). Porewater ammonia concentrations in all samples were < 20 mg/L and no mitigation measures were taken. Bioassays for each species were initiated using 8 laboratory replicates (100 mL sediment and 175 mL overlying water) from each sample into which 10 test organisms were added. At the time of test initiation, *C. dilutus* were approximately nine days old with an average ash-free dry weight of 0.220 mg and *H. azteca* were 11–12 days old with an average dry weight of 0.022 mg. At the conclusion of the 10-day exposure, the percentage of surviving organisms (hereafter "survival") and the average weight (ash-free dry weight for *C. dilutus* and dry weight for *H. azteca*) of the surviving organisms (hereafter "growth") were assessed for each replicate (USEPA, 2000). The quality of the data generated by the toxicity tests was assured by: (a) testing two laboratory control samples (control 1: clean sediment and overlying water; control 2: water only) and (b) daily monitoring of temperature and dissolved oxygen in overlying water to verify that test conditions and organism responses met test acceptability criteria (USEPA, 2000). Additionally, a duplicate sample from one site was collected and analyzed to assess the precision of test endpoints.

2.3. Habitat characterization

A standard suite of habitat and water-quality parameters were measured at each site to evaluate habitat comparability between sites and potential influences on community composition and sediment toxicity tests. Water quality parameters including specific conductance, dissolved oxygen, pH, and temperature were measured at 1 m above the river bottom at the time of sample collection using a YSI Professional Plus Multiparameter Water Quality Meter following quality assurance and calibration procedures described in NYSDEC (2023b). Grain size was characterized using the ASTM D422–63 method (ASTM, 2007) for determining the distribution of particle sizes. The percentage of each sample that was composed of fine sediments (the silt and clay fractions), was calculated as percent by mass able to pass through a No. 200 (75 μm) sieve. Total organic carbon was measured using method 9060A (USEPA, 2004) and is reported here as a percentage of the total sample by dry weight. The raw data from macroinvertebrate identification, sediment toxicity tests, and grain-size and total organic carbon analyses are available in a USGS data release (George and Baldigo, 2022).

2.4. Statistical analysis

A preliminary inspection of the macroinvertebrate identification data indicated that 30 of the 42 community replicates (71%) did not reach the target subsample size of 100-organisms despite the large quantity of sediment processed. Thus, two different approaches were used to calculate BAP scores from this dataset. For the first approach, differences in subsample sizes were ignored and BAP scores were calculated for all replicates following standard procedures described in Duffy (2021) and are presented as the average of the scores from the three replicate samples. For the second approach, a technique described in George et al. (2017) was used in which the data from all three replicates were combined for each site and then rarefied (without replacement) to produce a random 100-count subsample. This procedure was repeated for 30 consecutive iterations and BAP scores were then calculated and are presented as the mean score of those 30 random subsamples for each site. The former approach represents a consistent level of sampling effort, accounts for the density of organisms present, and follows standard NYSDEC protocols (Duffy, 2021). The latter approach, by simulating the 100-organism target count, provides an assessment that may be more appropriate for evaluating the integrity of macroinvertebrate communities relative to the established NYSDEC impact categories and BUI removal criteria. Hereafter, the results of the first approach are referred to as 'standard BAP scores' and the results of the second approach are termed 'aggregate BAP scores'.

The standard BAP scores, aggregate BAP scores, and endpoints from the toxicity tests were compared between the AOC and reference areas using a noninferiority testing framework. This type of statistical approach reverses the typical hypothesis testing structure and puts the burden of proof on demonstrating equivalence, rather than difference (Lakens, 2017; Mascha and Sessler, 2011; Walker and Nowacki, 2011). Although the results of this approach can be more difficult to communicate to managers and stakeholders, this type of analysis is warranted in the AOC-framework where the goal of management action is to restore the condition of an impacted area to that of the surrounding area (Rutter, 2010). This type of hypothesis testing is particularly necessary

for evaluating the specific removal criteria for the benthos BUI in the Eighteenmile Creek AOC which state that community condition and sediment toxicity should be "similar" to reference sites. One of the main challenges with this type of analysis is determining the degree of departure from the reference condition that is acceptable. There is little consensus regarding the effect size that monitoring programs should target for detecting change in aquatic assemblages (Janz et al., 2010). Consequently in this analysis, 25% was used because it has been recommended as appropriate for many ecological monitoring applications (Munkittrick et al., 2009). Noninferiority testing was performed using one-sided, two-sample equivalence tests (not assuming equal variance) using Minitab v17. Noninferiority was established only if the entire 95% confidence interval around the ratio of the test mean (AOC) to the reference mean (Oak Orchard Creek) was greater than a lower limit of 0.75 (i.e., establish 95% confidence that the test mean was at least 75% of the reference mean).

The structure of macroinvertebrate communities was also evaluated using multivariate techniques with PRIMER-E v7 software (Clarke and Gorley, 2015). Although such analyses do not produce output directly related to the BUI removal criteria, they provide a robust assessment of community composition and can identify key taxa or groups of taxa responsible for patterns in the dataset. The raw taxa counts from all three replicates were summed for each sample, square-root transformed, and used to form a resemblance matrix of Bray-Curtis similarities comparing all samples. A non-metric multidimensional scaling (nMDS) ordination was used to plot the Bray-Curtis similarities and visually assess differences in macroinvertebrate community structure between sites (Clarke and Gorley, 2015; Clarke et al., 2014). Similarity percentages (SIMPER) analysis was then used to identify the taxa that contributed most strongly to any observed differences in the composition of communities between the AOC and reference area.

3. Results

3.1. Macroinvertebrate communities

Macroinvertebrate communities in the Eighteenmile Creek AOC were composed of organisms from 15 taxonomic orders and were dominated by chironomids in the order Diptera (primarily genera *Procladius* and *Chironomus*). Macroinvertebrate communities in the Oak Orchard Creek reference area were composed of organisms from nine taxonomic orders and were also dominated by chironomid-family Diptera (primarily genus *Procladius*). Eight orders were present in both the AOC and reference area, while seven orders were found exclusively in the AOC (Amphipoda, Basommatophora, Hoplonemertea, Lumbriculida, Megaloptera, Odonata, and Trichoptera) and one order (Coleoptera) was found exclusively in the reference area (Table 2). Within the sensitive EPT (Ephemeroptera, Plecoptera, Trichoptera) orders, Ephemeroptera (mayfly larvae) were present at six AOC sites, reaching a peak relative abundance of 13.0% at emil-2.5, Plecoptera (stonefly larvae) were not found at any AOC site, and Trichoptera (caddisfly larvae) were present at two AOC sites reaching a peak relative abundance of 3.0% at emil-0.6 (Table 2). In the reference area, Ephemeroptera were found at the four upstream-most sites, reaching a peak relative abundance of 4.5% at oak-5.9, while Trichoptera and Plecoptera were not found at any reference site.

Multivariate analyses indicated the composition of macroinvertebrate communities differed considerably between the AOC and reference area. The nMDS ordination revealed tight clustering of the reference sites while the AOC sites exhibited more within-group dissimilarly and were widely distributed through ordination space (Fig. 2). The similarity percentages analysis indicated the three most discriminating taxa responsible for differences between AOC and reference sites were 'undetermined Tubificidae without cap. setae' (Tubificidae) and *Paralauterborniella nigrohalteralis* (Chironomidae) which both had considerably greater abundance in the reference area, and genus *Chironomus* (Chironomidae) which had greater abundance in the AOC.

The standard and aggregate BAP scores indicated that community condition was similar between the AOC and reference area but considerably more variability was observed between sites in the AOC. The standard BAP scores at AOC sites ranged from 2.2 at emil-1.6 to 8.1 at emil-2.5 and averaged 5.1, compared to the reference area where scores ranged from 3.9 at oak-0.2 and oak-3.9 to 6.0 at oak-2.5 and averaged 4.8 (Table 3). Standard BAP scores indicated that one of the eight AOC sites was classified as severely impacted, three of eight were moderately impacted, three of eight were slightly impacted, and one of eight was non-impacted, whereas four of the six reference sites were classified as moderately impacted and two of six were slightly impacted (Fig. 3). The aggregate BAP scores at AOC sites ranged from 2.7 at emil-1.6 to 8.7 at emil-2.3 and averaged 6.6, compared to the reference area where scores ranged from 4.0 at oak-0.2 to 6.8 at oak-2.5 and averaged 5.5 (Table 3). Aggregate BAP scores indicated that two of the eight AOC sites were moderately impacted, three of eight were slightly impacted, and three of eight were non-impacted; two of the six reference sites were classified as moderately impacted and four of six were slightly impacted (Fig. 3). Noninferiority was established for the AOC with both the standard BAP ($T = 1.905$, $P = 0.045$, $df = 9$) and aggregate BAP ($T = 3.102$, $P = 0.005$, $df = 11$) as the lower bound of the 95% confidence interval about the ratio of the Eighteenmile Creek and Oak Orchard Creek means did not

Table 2
Percent contribution (relative abundance) of all taxonomic orders from eight sites in the Eighteenmile Creek AOC and six sites in the Oak Orchard Creek reference area where macroinvertebrate communities were sampled in 2021. Percentages were calculated by summing the counts of all taxa from the three replicates at each site (George and Baldigo, 2022) and then determining the relative abundance of each taxonomic order to the entire sample.

	emil-0.2	emil-0.6	emil-1.0	emil-1.6	emil-1.9	emil-2.2	emil-2.3	emil-2.5	oak-0.2	oak-1.8	oak-2.5	oak-3.3	oak-3.9	oak-5.9
Amphipoda	26.9	12.6	0.8	–	1.3	2.1	5.1	24.0	–	–	–	–	–	–
Basommatophora	–	4.8	–	–	0.7	–	0.6	–	–	–	–	–	–	–
Coleoptera	–	–	–	–	–	–	–	–	–	–	0.3	–	–	0.6
Diptera	50.3	65.3	77.4	94.4	73.6	87.6	38.0	44.0	55.1	74.5	71.7	78.0	77.1	77.6
Ephemeroptera	1.2	1.2	–	–	7.4	1.0	11.4	13.0	–	–	0.3	1.0	0.5	4.5
Hirudinida	0.6	0.6	19.4	4.0	4.7	–	9.5	2.7	–	0.5	0.7	–	–	–
Hoplonemertea	–	–	–	–	–	–	1.3	–	–	–	–	–	–	–
Isopoda	0.6	–	–	–	0.7	–	13.3	4.0	–	–	–	–	–	0.6
Lumbriculida	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–
Megaloptera	–	4.8	–	–	–	–	–	–	–	–	–	–	–	–
Mesogastropoda	1.8	4.2	–	0.8	2.0	–	0.6	–	1.8	–	0.3	0.5	–	–
Odonata	0.6	1.2	–	0.8	1.0	–	1.9	1.7	–	–	–	–	–	–
Trichoptera	–	3.0	–	–	–	–	1.3	–	–	–	–	–	–	–
Tubificida	13.5	1.8	2.4	–	8.7	9.3	17.1	10.0	42.5	25.0	26.0	20.5	22.4	16.0
Unionida	–	–	–	–	–	–	–	0.3	–	–	0.3	–	–	–
Veneroida	4.7	0.6	–	–	–	–	–	–	0.7	–	0.3	–	–	0.6

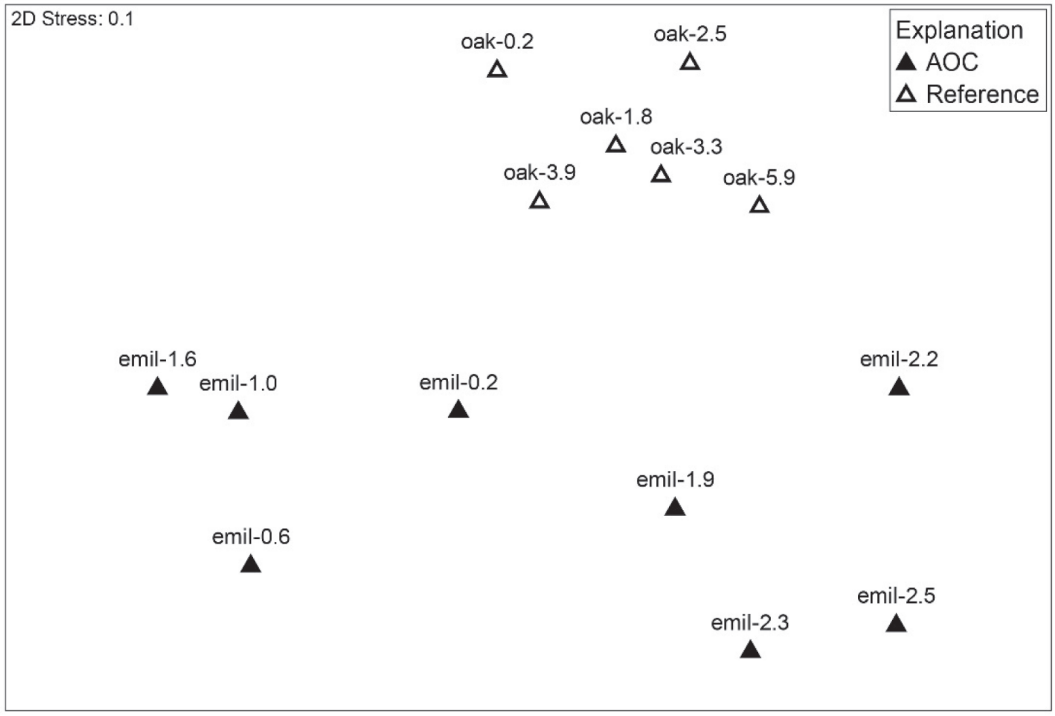

Fig. 2. Non-metric multidimensional scaling (nMDS) ordination of macroinvertebrate community composition. The ordination plots Bray-Curtis similarities derived from square-root transformed community data summed across the three replicates for each sample.

Table 3
Macroinvertebrate community information including subsample size, component metrics of the Biological Assessment Profile (BAP) score, and the final (10-scaled) standard BAP score presented as mean (standard error) for the three replicates collected at each site (George and Baldigo, 2022). The aggregate BAP score presents the mean (standard error) BAP score of 30 random 100-organism subsamples from the combined replicates at each site.

Site ID	Subsample size (no. of organisms)	Species richness	Hilsenhoff Biotic Index	Percent model affinity	Shannon Diversity Index	Dominant-3	Standard BAP score	Aggregate BAP score
emil-0.2	57 (26)	11.3 (3.7)	8.2 (0.3)	47.0 (7.2)	2.5 (0.4)	76.6 (8.6)	4.2 (1.3)	6.5 (0.1)
emil-0.6	56 (24)	10.7 (4.8)	8.2 (0.6)	48.7 (12.9)	2.4 (0.6)	75.6 (13.3)	4.1 (2.2)	6.9 (0.1)
emil-1.0	41 (15)	6.0 (2.1)	7.8 (0.6)	41.7 (7.6)	1.9 (0.5)	83.3 (8.3)	3.0 (0.7)	4.6 (0.0)
emil-1.6	42 (5)	7.0 (0.6)	8.9 (0.1)	37.3 (3.8)	2.1 (0.1)	85.1 (2.0)	2.2 (0.4)	2.7 (0.1)
emil-1.9	100 (0)	22.3 (0.7)	7.8 (0.4)	47.3 (8.3)	3.6 (0.1)	50.5 (1.8)	7.2 (0.3)	7.5 (0.1)
emil-2.2	32 (5)	11.7 (1.3)	7.2 (0.2)	39.0 (4.7)	3.1 (0.1)	56.4 (3.3)	5.6 (0.6)	7.3 (0.0)
emil-2.3	53 (19)	16.0 (6.2)	8.0 (0.1)	68.3 (9.2)	3.1 (0.6)	57.8 (12.0)	6.4 (1.7)	8.7 (0.0)
emil-2.5	100 (0)	24.0 (1.7)	7.7 (0.2)	64.0 (1.2)	3.8 (0.2)	48.7 (4.5)	8.1 (0.3)	8.4 (0.0)
oak-0.2	95 (5)	13.0 (0.6)	9.3 (0.1)	42.3 (1.5)	2.6 (0.1)	73.7 (2.0)	3.9 (0.3)	4.0 (0.1)
oak-1.8	61 (10)	14.3 (2.6)	8.7 (0.3)	35.7 (4.3)	3.1 (0.2)	59.4 (2.9)	4.9 (0.5)	5.9 (0.0)
oak-2.5	100 (0)	17.0 (0.6)	8.0 (0.2)	41.0 (1.5)	3.3 (0.0)	56.3 (1.5)	6.0 (0.1)	6.8 (0.0)
oak-3.3	67 (11)	11.7 (1.5)	8.2 (0.1)	40.3 (2.2)	3.0 (0.1)	58.9 (3.5)	4.9 (0.4)	5.6 (0.1)
oak-3.9	64 (11)	12.0 (0.6)	8.8 (0.1)	38.0 (1.2)	2.6 (0.1)	69.9 (0.6)	3.9 (0.1)	4.3 (0.1)
oak-5.9	52 (14)	12.7 (1.5)	8.1 (0.3)	40.0 (3.0)	3.2 (0.1)	55.0 (1.0)	5.4 (0.3)	6.4 (0.0)

extend below the threshold value of 0.75 for either metric.

3.2. Sediment toxicity test results

The survival and growth of *C. dilutus* exceeded the minimum test acceptability criteria of 70% and 0.48 mg (USEPA, 2000), respectively, in both laboratory controls. Similarly, *H. azteca* met the minimum test acceptability criteria of 80% survival and exhibited measurable growth in both laboratory controls. The daily measurements of overlying water quality were all within the acceptable ranges (temperature: 23 °C ± 1, dissolved oxygen > 2.5 mg/L) for each test method with no deviations observed (USEPA, 2000). Similarly, parameters measured at test initiation and termination (hardness, alkalinity, ammonia, pH, and conductivity) exhibited negligible variability and met the test acceptability criteria of not varying by > 50% (USEPA, 2000). Toxicity test results from the duplicate sediment samples collected at emil-1.6 indicated high precision with the survival endpoint and more variability around the growth endpoint for both species. The relative percent difference between the duplicate samples was 4.0% for survival of *C. dilutus*, 11.8% for growth of *C. dilutus*, 0% for survival of *H. azteca*, and 29.2% for growth of *H. azteca*. Overall, these quality assurance data indicate that test acceptability criteria were met, and therefore, the test results can be considered valid assessments of sediment toxicity.

Survival and growth of *C. dilutus* and *H. azteca* were generally similar between the AOC and reference area (Fig. 4). A notable outlier in the dataset occurred with the *H. azteca* data from three reference sites where total or near-total mortality occurred. Survival and growth of *C. dilutus* averaged 94.4% and 1.23 mg, respectively, across sites in the AOC compared to an average of 95.0% and 1.03 mg across all reference sites (Table 4). Survival and growth of *H. azteca* averaged 97.5% and 0.13 mg, respectively, across sites in the AOC compared to an average of 52.3% and 0.08 mg across all reference sites (Table 4). Noninferiority was established for the AOC with both *C. dilutus* endpoints (survival: $T = 18.753$, $P < 0.001$, $df = 11$, growth: $T = 5.862$, $P < 0.001$, $df = 11$) and

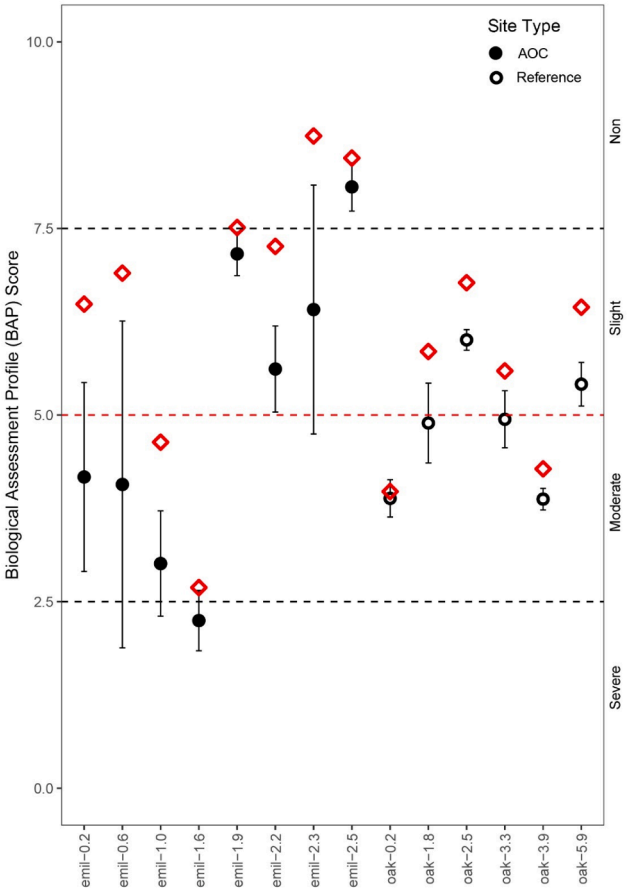

Fig. 3. Biological Assessment Profile (BAP) scores of macroinvertebrate community integrity shown in black as the standard BAP score (mean ± one standard error, n = 3) and in red as the aggregate BAP score for eight sites in the Eighteenmile Creek AOC and six sites in the Oak Orchard Creek reference area. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

both *H. azteca* endpoints (survival: T = 3.764, P = 0.007, df = 5, growth: T = 2.882, P = 0.017, df = 5).

4. Discussion

The primary objective of this study was to assess the current status of macroinvertebrate communities and sediment toxicity in the Eighteenmile Creek AOC to determine whether legacy sediment contamination is causing impairment to the benthic component of the aquatic ecosystem. The results from macroinvertebrate community assessment at eight sites in the AOC during 2021 indicate that community condition spanned a wide range, but the mean condition was similar to that of a reference area on Oak Orchard Creek. Additionally, sediment toxicity tests using *C. dilutus* and *H. azteca* found no evidence that bed sediments in the AOC caused toxicity to either test species. The results of non-inferiority tests indicated that the standard and aggregate BAP scores, as well as the survival and growth endpoints for both toxicity-test species, were not inferior in the AOC relative to the reference area. For most of these comparisons, the mean value of the metric or endpoint in the AOC was higher than the corresponding value from the reference area. Despite these largely positive findings, there were several noteworthy patterns in the data, and informative comparisons with prior data from this AOC and from other systems, that warrant further discussion.

The condition and composition of macroinvertebrate communities in the AOC were considerably more variable than that observed in the reference area. This increased variability in the AOC was evident both between sites, where standard BAP scores ranged from 2.2 to 8.1 among AOC sites compared with 3.9 to 6.0 among reference sites, and within sites where variability between the three replicates was markedly higher at the AOC sites compared to the reference sites as shown by the standard error bars in Fig. 3. This indicates that at both the minute spatial scale of 1–2 m between replicates within a site, and at the broad spatial scale of both systems (multiple kilometers), the condition of macroinvertebrate communities was far more variable in the AOC than the reference area. Some of the within-site variability may be attributable to large differences in subsample sizes between replicates at some AOC sites. For example, at site emil-0.6 where the standard BAP scores ranged widely between the three replicates, the number of organisms obtained from the detritus of the three respective replicates was 100, 16, and 51 (George and Baldigo, 2022). However, even when the three replicates were pooled for each site in the multivariate analysis, the nMDS ordination showed that the structure or composition of communities varied considerably between AOC sites while the six reference sites grouped closely together. Without additional sampling and comprehensive comparisons of habitat heterogeneity between the AOC and reference area, it is not possible to confidently determine if the increased variability observed in macroinvertebrate communities at AOC sites is an indicator of stress. Despite the uncertainty as to the source of the variability, the removal criteria for the benthos BUI do not require direct consideration of variability.

The high mortality of *H. azteca* at three of the six reference sites was unexpected and difficult to interpret but does not alter the overall conclusions from this study or prohibit an assessment of whether the benthos BUI removal criteria have been met. Exposure to sediments from the three downstream-most reference sites, oak-0.2. oak-1.8, and oak-2.5, caused total or near-total mortality of *H. azteca*, while comparable *C. dilutus* tests from the same sites indicated no effect on survival and potentially a slight reduction of growth. All associated laboratory controls indicated that the starting batch of test organisms was healthy, and daily monitoring of overlying water in the test chambers found no deviations of temperature or dissolved oxygen outside the test acceptability criteria (USEPA, 2000). Similarly, porewater ammonia concentrations were within the ranges observed in other samples and were well below the 20 mg/L threshold that would require mitigation (USEPA and USACE, 1998a). There was also no evidence that indigenous organisms were responsible for the mortality as the 100 mL-sediment aliquots used in each test chamber were visually screened for their presence during test initiation and test takedown. Habitat data collected from the three sites including particle size, TOC, depth, and dissolved oxygen also were not atypical and were within ranges observed at the other AOC and reference sites (Table 1). The NYSDEC Division of Environmental Remediation's Spill Incidents Database (NYSDEC, 2023a) was also queried to investigate if the toxicity observed at the three downstream-most reference sites could be the result of a known discharge. There were no records of chemical or petroleum spill incidents impacting the lower section of Oak Orchard Creek in the 10 years prior to this study. Thus, the most obvious explanation for this mortality is the presence of some unidentified stressor or contaminant in the sediment to which *H. azteca* has greater sensitivity than *C. dilutus*. However, the limited sediment chemistry data available from Oak Orchard Creek does not provide any additional insight into a possible cause. Analysis of a sediment sample collected in 2019 from an area near the reference sites in this study with *H. azteca* mortality did not detect the presence of PCB Aroclors, PAHs, or pesticides (WSP, 2021, 2022). Metals were detected, but not at concentrations exceeding NYSDEC Class A screening criteria (indicating little or no potential risk to aquatic life) (NYSDEC, 2014), and acid volatile sulfide / simultaneously extracted metals (AVS/SEM) analysis suggested that metals at this site were unlikely to be bioavailable. Three additional sediment samples collected from the same area on Oak Orchard Creek in 2020 yielded similar results (WSP, 2022). Additionally, prior sediment toxicity testing in Oak Orchard Creek has consistently

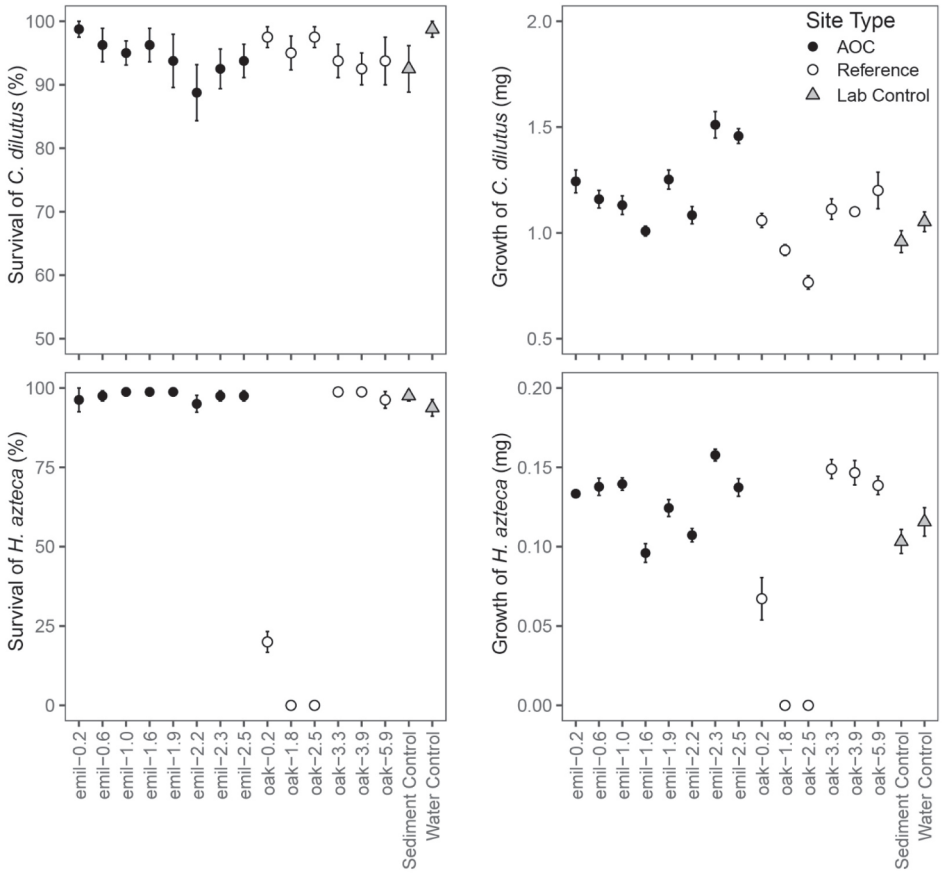

Fig. 4. Interval plots (mean ± one standard error, n = 8) showing the survival and growth of *C. dilutus* and *H. azteca* in 10-day sediment toxicity tests from study sites and laboratory controls.

Table 4
Results from 10-day sediment toxicity tests (George and Baldigo, 2022) presented as mean (standard error) for survival and growth of *C. dilutus* and *H. azteca* from the eight test replicates conducted for each field sample and laboratory control.

Site ID	*C. dilutus* survival (%)	*C. dilutus* growth (mg)	*H. azteca* survival (%)	*H. azteca* growth (mg)
emil-0.2	98.8 (1.2)	1.243 (0.054)	96.3 (3.7)	0.133 (0.002)
emil-0.6	96.3 (2.6)	1.159 (0.042)	97.5 (1.6)	0.138 (0.005)
emil-1.0	95.0 (1.9)	1.131 (0.044)	98.8 (1.2)	0.139 (0.004)
emil-1.6	96.3 (2.6)	1.008 (0.024)	98.8 (1.2)	0.096 (0.006)
emil-1.9	93.8 (4.2)	1.252 (0.045)	98.8 (1.2)	0.124 (0.005)
emil-2.2	88.8 (4.4)	1.083 (0.041)	95.0 (2.7)	0.107 (0.004)
emil-2.3	92.5 (3.1)	1.511 (0.063)	97.5 (1.6)	0.158 (0.004)
emil-2.5	93.8 (2.6)	1.457 (0.035)	97.5 (1.6)	0.137 (0.006)
oak-0.2	97.5 (1.6)	1.059 (0.033)	20.0 (3.3)	0.067 (0.013)
oak-1.8	95.0 (2.7)	0.918 (0.026)	0.0 (0)	no survival
oak-2.5	97.5 (1.6)	0.766 (0.032)	0.0 (0)	no survival
oak-3.3	93.8 (2.6)	1.112 (0.049)	98.8 (1.2)	0.149 (0.006)
oak-3.9	92.5 (2.5)	1.100 (0.018)	98.8 (1.2)	0.147 (0.008)
oak-5.9	93.8 (3.7)	1.200 (0.087)	96.3 (2.6)	0.139 (0.006)
Duplicate (emil-1.6)	92.5 (2.5)	1.134 (0.057)	98.8 (1.2)	0.129 (0.004)
Lab control (sediment and water)	92.5 (3.7)	0.959 (0.052)	97.5 (1.6)	0.103 (0.007)
Lab control (water only)	98.8 (1.2)	1.052 (0.047)	93.8 (2.6)	0.116 (0.009)

found no evidence of toxicity to *C. dilutus* and *H. azteca* (George et al., 2017; WSP, 2021) and numerous past and ongoing efforts in the AOC and Superfund programs have deemed it a suitable reference area indicative of typical regional conditions (E&E, 2009, 2017, 2019; George et al., 2022a).

Despite the challenges posed by the unexplained mortality of *H. azteca* in sediments from three reference sites, numerous lines of evidence support the conclusion that sediments in the AOC are not toxic to benthic macroinvertebrates. First, if the three anomalous reference sites are removed from the comparisons of the toxicity endpoints, the mean values from the three remaining reference sites were nearly identical to the corresponding endpoints from the eight AOC sites. Conversely, if these results were not excluded, most analyses would indicate that sediments from the AOC were less toxic than those from the reference area. Second, none of the AOC sites met the USEPA and U.S. Army Corps of Engineers standard criteria for toxicity used for sediment disposal decisions (USEPA and USACE, 1998b). These criteria state that sediments are considered to be toxic if any of the following criteria are met:

-mortality of *C. dilutus* > 20% higher than in reference sediments and

difference is statistically significant, OR

-mortality of *H. azetca* > 10% higher than in reference sediments and difference is statistically significant, OR

-mean dry weight (growth) of *C. dilutus* < 0.6 mg per organism, and difference between test and reference sediments > 10%, and difference is statistically significant

None of the eight AOC sites in this study met, or even approached, these criteria regardless of whether the Oak Orchard Creek reference sites or the laboratory test controls were used for the comparison. Finally, the results of the 10-day toxicity tests from the eight AOC sites in Eighteenmile Creek were generally similar to or showed less toxicity than results from other reference areas used in past AOC assessments in New York. The survival of *C. dilutus*, growth of *C. dilutus*, survival of *H. azteca*, and growth of *H. azteca* at the eight sites in the Eighteenmile Creek AOC averaged 94.4%, 1.23 mg, 97.5%, and 0.13 mg, respectively. Comparable data for the four toxicity endpoints from six reference sites on the Buffalo River averaged 82.9%, 1.58 mg, 97.1% and 0.07 mg, respectively in 2017, and 97.0%, 1.37 mg, 93.3% and 0.13 mg, respectively in 2020 (George et al., 2022b). Similarly, at ten reference sites on the upper Niagara River sampled in 2019, the toxicity endpoints averaged 84.6%, 1.64 mg, 95.2%, 0.12 mg, respectively (Baldigo et al., 2023). Thus, the toxicity test results from the eight sites in the Eighteenmile Creek AOC sampled in 2021 appear to be consistent with regional reference conditions and provide no indication that sediments were toxic to macroinvertebrates.

While it is difficult to ascertain with much confidence, the macroinvertebrate data collected in Eighteenmile Creek during 2021 and in prior efforts generally indicate that benthic communities have not changed much or improved only slightly over the past three decades. It is challenging to assess changes in community condition and structure over time due to inconsistent sampling methods, locations, and number of sites, and levels of taxonomic resolution between datasets, but some comparisons are possible. The percentage of communities (relative abundance) composed of chironomids (non-biting midges) and oligochaetes (worms), which are generally considered to be pollution tolerant, was 83.4% at one location sampled with a standard Ponar in 1994 (Abele et al., 1998), compared to an average of 86.2% from three petite Ponar sites in 2012 (E&E, 2013), 92.7% from three petite Ponar sites in 2014 (George et al., 2017), and 66.9% from the eight petite Ponar sites in 2021 described in this study (George and Baldigo, 2022). In a similar comparison of the sensitive EPT orders, the relative abundance of mayfly, stonefly, and caddisfly larvae was 0.0, 0.0, and 0.6% in the 1994 dataset, 1.4, 0.2, and 1.6% in the 2012 data, 0.0, 0.0, 0.8% in the 2014 data, and 4.4, 0.0, 0.5% in the 2021 data. Standard and aggregate BAP scores from the three 2014 samples averaged 3.8 and 5.9 respectively (George et al., 2017), compared to 4.3 and 6.1, at the same three sites resampled in 2021 as part of this study (emil-0.2, emil-1.0, and emil-2.2). Together, these data indicate that the condition of macroinvertebrate communities has remained fairly static over the past three decades.

The results from this assessment of macroinvertebrate community condition and sediment toxicity, when interpreted in conjunction with other studies described above, have important implications for assessing the status of the benthos BUI in the Eighteenmile Creek AOC. The mean condition of macroinvertebrate communities, as calculated using both the standard BAP and aggregate BAP score, was 5.1 and 6.6 respectively, thus falling into the slightly impacted category and meeting the first criterion of being '*"non-impacted" or "slightly impacted" according to NYSDEC indices*'. Although the mean condition met this criterion, four and two of the eight individual AOC sites, using the two respective indices, did not meet this criterion. Thus, it may be appropriate to consider the second and third criterion, which together, require that community condition and sediment toxicity in the AOC be similar to that of a comparable reference area. The data collected during this study and corresponding noninferiority tests indicate that condition of macroinvertebrate communities and the quality of sediments in the AOC were similar or potentially superior to that of the reference area. These results corroborate findings from three recent studies which produced similar conclusions (George et al., 2017; WSP, 2022; E&E, 2013). Therefore, the weight of evidence from the existing suite of data from all sources indicates that benthic macroinvertebrate communities in the Eighteenmile Creek AOC are similar to the regional condition and are not impaired by chemical contamination of sediments. While the decision whether to remove the benthos BUI ultimately lies with the Eighteenmile Creek AOC Remedial Advisory Committee and associated state and federal agencies, the findings presented in this manuscript are an important contribution towards that action and for informing approaches to assessing this BUI in other AOCs across the Great Lakes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Assessing the status of sediment toxicity and macroinvertebrate communities in the Eighteenmile Creek Area of Concern, New York

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ABSTRACT

In 1972, the governments of Canada and the United States committed to restoring the physical, chemical, and biological integrity of the Laurentian Great Lakes under the Great Lakes Water Quality Agreement. Through this framework, the downstream-most section of Eighteenmile Creek, a tributary to the south shore of Lake Ontario in New York, was designated as an Area of Concern (AOC) because water quality and bed sediments were contaminated by past industrial and municipal discharges, waste disposal, and pesticide usage. Five beneficial use impairments (BUIs) have been identified in the AOC including the degradation of the "benthos", or the benthic macroinvertebrate community. This investigation used sediment toxicity testing and macroinvertebrate community assessments to determine if the toxicity of bed sediments in the AOC differed from that of an unimpacted reference stream. Results from 10-day toxicity tests indicated that survival and growth of the dipteran *Chironomus dilutus* and the amphipod *Hyalella azteca* did not differ significantly between sediments from the AOC and reference area. Analyses of benthic macroinvertebrate community integrity and structure also indicated that macroinvertebrate communities, while impacted across most sites on both streams, were generally similar between the AOC and reference area. Despite these findings, the upstream-most AOC site consistently scored poorly in all analyses, which suggests that localized sediment toxicity may exist in the AOC, even if large scale differences between the AOC and a comparable reference stream are minimal.

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Introduction

In 1972, the governments of Canada and the United States committed to restoring the physical, chemical, and biological integrity of the Laurentian Great Lakes under the Great Lakes Water Quality Agreement (GLWQA). The purpose of this agreement, its successor agreement in 1978, and subsequent amendments, was to provide a framework for binational cooperation to restore, protect, and enhance the water quality of the Great Lakes in order to promote the ecological health of the Great Lakes basin (GLWQA, 2012). Through this framework, 43 Areas of Concern (AOCs) were subsequently identified in the Great Lakes basin. Areas of Concern are defined as geographic areas impacted by environmental degradation resulting from human activities at the local level, and exhibit impairment to one or more of 14 possible beneficial uses relating to chemical, physical, or biological integrity. For each AOC, a Remedial Action Plan is developed by a local remedial action committee to guide restoration efforts and the evaluation of recovery. Beneficial use impairments (BUIs) are then reevaluated over time, or following remedial efforts, to determine if they are still applicable to an AOC or if the BUIs may be removed and the entire AOC delisted.

Eighteenmile Creek, located in Niagara County of New York State, was designated as an AOC in 1985 because water quality and bed sediments were contaminated by past industrial and municipal discharges, waste disposal, and pesticide usage (CH2MHILL et al., 2015; NCSWCD, 2011; NYSDOH, 2015). In 2012, the AOC and areas upstream of it were also added to the Superfund National Priorities List of the country's most hazardous waste sites (USEPA, 2012b). Five BUIs have been identified in the Eighteenmile Creek AOC, including the degradation of the "benthos", or the benthic macroinvertebrate community. Assessments conducted by the U.S. Army Corps of Engineers and the New York State Department of Environmental Conservation (NYSDEC) between 1977 and 1994, which indicated that macroinvertebrate communities were adversely affected by contaminated surficial sediments, provided the rationale for this BUI (NCSWCD, 2011). The current status of the benthos BUI needs to be updated, however, because new inputs of contaminants have been largely eliminated (NCSWCD, 2011) and data from one recent investigation suggests that macroinvertebrate communities in the Eighteenmile Creek AOC may no longer be impaired (E&E, 2013).

The U.S. Geological Survey (USGS) and NYSDEC initiated the current study during 2014 to gather more extensive information on the toxicity

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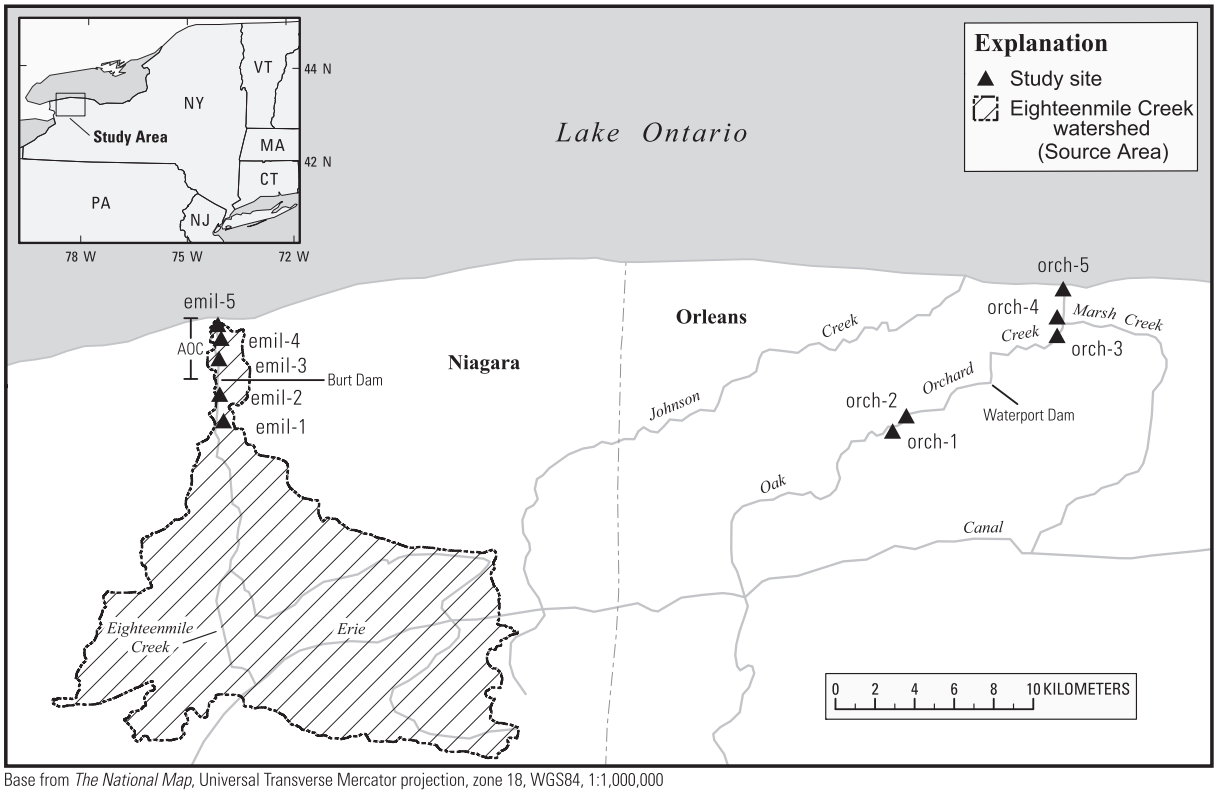
of sediments and condition of benthic macroinvertebrate communities needed to evaluate the benthos BUI in the Eighteenmile Creek AOC. The general delisting guidelines from the International Joint Commission (IJC), an independent binational organization charged with implementing the GLWQA, state that the benthos BUI may be removed from an AOC when benthic macroinvertebrate community structure or sediment toxicity do not differ from comparable unimpacted reference sites (IJC, 1991). Additionally, the Remedial Action Plan for the Eighteenmile Creek AOC provides specific criteria for removing the benthos BUI, most notably that benthic macroinvertebrate communities be classified as non-impacted or slightly impacted according to NYSDEC macroinvertebrate indices (NCSWCD, 2011). The primary objective of this study was to determine if the benthos BUI is still warranted in the Eighteenmile Creek AOC as defined by both the IJC guidelines and the Remedial Action Plan criteria. This was achieved by comparing the results of (a) laboratory sediment toxicity tests (survival and growth of two benthic macroinvertebrate species) and (b) benthic macroinvertebrate community assessments, at sites located within the AOC to reference sites located outside the AOC. This approach of assessing difference from comparable reference conditions is suggested by Grapentine (2009) and has been used in several other BUI assessments conducted in New York (Baldigo et al., 2012; Baldigo et al., 2016; Duffy et al., 2016) because it helps control for confounding regional stressors such as eutrophication and sedimentation. It is also consistent with a guidance document provided by the NYSDEC which describes the goal of the AOC remedial process in New York State as ensuring that conditions in an AOC are no worse than those in the surrounding area (NYSDEC, 2010).

Methods

Study area

The main branch of Eighteenmile Creek is approximately 24 km long and flows north from its headwaters near Lockport to its mouth at Lake Ontario in Olcott, N.Y. The AOC is defined as the downstream-most section, specifically the 3.5-km reach between a hydroelectric dam (Burt Dam) and Lake Ontario (Fig. 1). Additionally, the entire Eighteenmile Creek watershed has been designated as the source area of the contaminants that degraded the quality of sediments in the AOC because most point sources of sediment contamination were located upstream of the AOC (CH2MHILL et al., 2015; E&E, 2007; Makarewicz and Lewis, 2010; NCSWCD, 2011). Polychlorinated biphenyls (PCBs), chlorinated pesticides, and a number of heavy metals have been found in bed sediments at concentrations well above NYSDEC standards both inside and upstream of the AOC (CH2MHILL et al., 2015; NCSWCD, 2011; NYSDOH, 2015; Pickard, 2006; Stackelberg and Gustavson, 2012), and these contaminants are believed to be the primary cause of impairment to the macroinvertebrate community. Within the AOC, the highest concentrations of most toxic substances were found in the upstream-most 2 km closest to Burt Dam, but surficial sediments throughout the AOC contain contaminant levels of toxicological concern (Pickard, 2006).

Stream habitats within the Eighteenmile Creek AOC range from approximately 30–90 m in width and 0.5–3.5 m in depth (E&E, 2003), and the annual mean discharge below Burt Dam was 5.3 m^3/s in 2014 (USGS, 2014). Sediment samples were collected from five sites on Eighteenmile Creek; three of which were located within the AOC and two of which were located in the impounded section of the source area upstream of Burt Dam (Table 1, Fig. 1). Additionally, sediment samples were collected from five reference sites on Oak Orchard Creek, a comparable stream that enters Lake Ontario approximately 43 km east of Eighteenmile Creek. Oak Orchard Creek is of similar size and surrounding geography, also has a hydroelectric dam (Waterport Dam), and has been used as a reference stream for the assessment of other BUIs in the Eighteenmile Creek AOC because it is not known to have contaminated bed sediments (E&E, 2009). For example, the fish tumors BUI for the Eighteenmile Creek AOC, originally listed as unknown, was evaluated and determined to be not impaired using reference data from Oak Orchard Creek (E&E, 2009). Similar evaluations (which did not result in BUI removal) were conducted for the fish and wildlife



Base from *The National Map*, Universal Transverse Mercator projection, zone 18, WGS84, 1:1,000,000

Fig. 1. Map of the study area showing five study sites on Eighteenmile Creek and five reference sites on Oak Orchard Creek where bed sediments were sampled.

Table 1
Site information, locations (latitude and longitude - NAD83), and habitat characteristics for sediment samples collected August 27–28, 2014 for analysis of sediment toxicity and macroinvertebrate communities.

Stream name	Site ID	Site type	Latitude	Longitude	Grain size (phi units)	% TOC
Eighteenmile Creek	emil-1	Source area	43.29416	−78.71191	5.91	4.82
Eighteenmile Creek	emil-2	Source area	43.30613	−78.71502	6.81	3.67
Eighteenmile Creek	emil-3	AOC	43.32229	−78.71644	4.24	2.63
Eighteenmile Creek	emil-4	AOC	43.33157	−78.71570	4.73	2.43
Eighteenmile Creek	emil-5	AOC	43.33779	−78.71810	5.39	3.40
Oak Orchard Creek	orch-1	Upstream reference	43.30223	−78.29538	4.55	1.99
Oak Orchard Creek	orch-2	Upstream reference	43.30942	−78.28720	6.45	3.30
Oak Orchard Creek	orch-3	Downstream reference	43.34861	−78.19528	4.43	0.78
Oak Orchard Creek	orch-4	Downstream reference	43.35707	−78.19573	4.57	1.90
Oak Orchard Creek	orch-5	Downstream reference	43.36994	−78.19263	5.42	2.41

populations BUI and the bird or mammal deformities or reproductive impairment BUI (E&E, 2009). Three sites on Oak Orchard Creek were located downstream of the Waterport Dam and two sites were located upstream of it to account for potential confounding effects of Burt Dam on macroinvertebrate communities in the Eighteenmile Creek AOC (Table 1, Fig. 1). Hereafter, the four site types will be referred to as source area (emil-1 and emil-2), AOC (emil-3, emil-4, and emil-5), upstream reference (orch-1 and orch-2), and downstream reference (orch-3, orch-4, and orch-5).

Sample collection and processing

Bed-sediment grab samples were collected from depositional areas at each site using a petite Ponar (0.03 m^2) dredge on August 27–28, 2014 for use in sediment toxicity tests, macroinvertebrate community assessment, and assessment of habitat comparability. For sediment toxicity tests, five grabs were collected from each site, composited and mixed in a bucket, and a 4-L subsample was stored in a polyethylene container. Samples were kept on ice and shipped to Great Lakes Environmental Center, Inc., Traverse City, MI, where testing was initiated within five weeks of sample collection. For habitat comparability, 0.24-L and 0.12-L subsamples were collected from the unused composite for measurement of grain-size distribution and total organic carbon (TOC), respectively, and shipped to ALS Environmental, Rochester, NY. For macroinvertebrate identification, five replicate samples were collected from each site. Each replicate was composed of the detritus from four composited grabs that were sieved through a 500 μm mesh screen bottom bucket, placed in a 1-L container, preserved with 95% ethanol, and shipped to Watershed Assessment Associates, Schenectady, NY.

Samples for sediment toxicity tests were used to quantify acute and chronic toxicity to the dipteran, *Chironomus dilutus* (10–11 days old at test initiation), and the amphipod, *Hyalella azteca* (9–10 days old at test initiation), during 10-day survival and growth bioassays following USEPA test methods 100.2 and 100.1, respectively (USEPA, 2000). *Chironomus dilutus* and *H. azteca* are used as indicator species because they each inhabit broad geographic ranges, burrow in sediments, and have known sensitivities to common nutrients and toxins (ASTM, 2010; USEPA, 2000; USEPA and USACE, 1998). In short, bioassays for each species were initiated using 8 laboratory replicates (100 mL sediment and 175 mL overlying water) from each sample into which 10 test organisms were added. At the conclusion of the 10-day exposure, the percentage of surviving organisms (hereafter "survival") and the average ash-free dry weight of the surviving organisms (hereafter "growth") were assessed for each replicate. The quality of the data generated by the toxicity tests was assured by (a) testing two laboratory control samples (control 1: clean sediment and overlying water; control 2: water only) and (b) daily monitoring of temperature and dissolved oxygen in overlying water to verify that test conditions and organism responses generally met test acceptability criteria (USEPA, 2000). Additionally, the precision of test endpoints was assessed using duplicate samples from two sites. USEPA test methods 100.2 and 100.1 (USEPA, 2000) provide a full summary of the test conditions and procedures used.

A 100-organism subsample, or an exhaustive pick when <100 organisms were present, was sorted from each macroinvertebrate community replicate using a gridded tray and identified to the lowest possible taxonomic resolution (usually genus or species). The NYSDEC multi-metric index of biological integrity for Ponar samples was then calculated to assess the condition of macroinvertebrate communities (Smith et al., 2014). The index calculates five component metrics: species richness, Hilsenhoff Biotic Index (Hilsenhoff, 1987), Dominant-3, Percent Model Affinity (Novak and Bode, 1992), and Shannon-Weiner diversity, and converts them to a standardized value on a scale from 0 to 10. The five component metrics are then averaged to produce a Biological Assessment Profile (BAP) score, a single value for which a four-tiered scale of water quality impact (severe: 0.0–2.5; moderate: 2.5–5.0; slight: 5.0–7.5; or non-impacted: 7.5–10.0) has been established (Smith et al., 2014). Impact tiers of moderate and severe are indicative of impaired conditions.

Grain size was characterized using the ASTM D422–63 method (ASTM, 2007) for determining the distribution of particle sizes. The mid-point of each particle-size class was converted to phi units (Cummins, 1962), weighted by percent contribution to the total, and summed for each sample to obtain a simplified grain-size distribution for comparing physical habitat differences between sites. TOC was measured using the Lloyd Kahn Method (Kahn, 1998) to determine if the productivity of sediments, as well as the potential for sediments to accumulate contaminants and make them biologically available, was similar between sites. Although sediment toxicity tests using *C. dilutus* and *H. azteca* may not be strongly affected by small differences in grain size and TOC (USEPA, 2000), a number of field studies have shown these variables can influence the structure of macroinvertebrate community assemblages (Breneman et al., 2000; Reinhold-Dudok and den Besten, 1999).

Statistical analysis

An exploratory analysis of the response variables (survival and growth of *C. dilutus*, survival and growth of *H. azteca*, and BAP score) was conducted using a linear mixed model in Minitab v17. The use of a linear mixed model provides a robust statistical framework in which the effects of one factor can be tested while controlling for the effects of others, and also allows for a hierarchical nesting structure that includes the individual replicates from each sample. The primary objective of this analysis was to determine if the three sites within the Eighteenmile Creek AOC differed from the three downstream reference sites on Oak Orchard Creek while accounting for natural differences between the two creeks and the sites selected on each creek. The model was constructed using three factors with the following nesting structure: stream, type (nested within stream), and site (nested within type). Stream and type were treated as fixed factors while site was

treated as a random factor in order to formulate broader conclusions about differences between site types rather than just the specific sites within each site type (Bolker et al., 2009). Histograms of the residuals and scatterplots of the fitted values versus the residuals were evaluated for each response variable to ensure there were no gross violations of normality or homoscedasticity. The results of all statistical analyses were considered significant at $\alpha = 0.05$ ($P \leq 0.05$).

Additionally, noninferiority testing using one-sided, one-sample equivalence tests was used to compare response variables at each of the three AOC sites to the mean value from the three downstream reference sites using Minitab v17. Noninferiority was established only if the entire 95% confidence interval around the difference between the test mean (average of the replicates of that site) and reference mean was greater than a lower limit of −0.2 multiplied by the reference mean (i.e. establish 95% confidence that the test mean was at least 80% of the reference mean). The use of 20% as a tolerance value is supported by numerous publications that have identified a 20% reduction in test sediments relative to control or reference sediments as a threshold for determining toxicity (Chapman and Anderson, 2005; Grapentine, 2009; USEPA, 2000, 2012a). Noninferiority testing improves the statistical inference of our analysis for two reasons. First, this approach enables a comparison of individual AOC sites to the mean reference condition, which was not possible using the linear mixed model while treating site as a random factor. Second, noninferiority testing puts the burden of proof on demonstrating equivalence, rather than difference (Mascha and Sessler, 2011; Walker and Nowacki, 2011). Such an approach is appropriate when the goal of management action is to restore the condition of an impacted area to that of the surrounding area and has recently been applied to the BUI-assessment framework (Rutter, 2010).

The structure of macroinvertebrate communities was also evaluated using multivariate techniques with PRIMER-E v7 software (Clarke and Gorley, 2015). The raw taxa counts from each replicate were $\log(x + 1)$ transformed and used to form a resemblance matrix of Bray-Curtis similarities. A one-way analysis of similarities (ANOSIM) test was used to assess differences in assemblages between the four site types (Clarke and Gorley, 2015; Clarke et al., 2014). Although the ANOSIM test produces a *P*-value, the value of the *R*-statistic is considered more important for assessing differences between groups (Clarke and Gorley, 2015). An *R* value of >0.75 indicates well separated groups, whereas an *R* value 0.5–0.75 indicates separate but abutting or slightly overlapping groups, and an *R* value of 0.25–0.5 indicates distinguishable but overlapping groups (K.R. Clarke, Plymouth Marine Laboratory, 2016, personal communication)(Ramette, 2007). Similarity percentages (SIMPER) analysis was then used to identify the taxa that contributed most strongly to observed differences between sites or site types. Additionally, a non-metric multidimensional scaling (nMDS) ordination plotting the Bray-Curtis similarities of combined (summing all replicates from each site) $\log(x + 1)$-transformed taxa counts was used to visually assess differences in macroinvertebrate community structure between sites and site types (Clarke and Gorley, 2015; Clarke et al., 2014).

Results and discussion

Sediment toxicity test quality assurance

The survival and growth of *C. dilutus* exceeded the minimum test acceptability criteria of 70% and 0.48 mg (USEPA, 2000), respectively, in both laboratory controls. Similarly, the survival and growth of *H. azteca* exceeded the minimum test acceptability criteria of 80% and exhibiting measurable growth, respectively, in both laboratory controls. The overlying water quality measurements were also within the acceptable limits for each test method with the exception of a few brief decreases in dissolved oxygen during *C. dilutus* tests, which are generally considered not to affect the quality of test data (USEPA, 2000). Toxicity test results from the two sets of duplicate sediment samples indicated that relative percent difference between duplicates was small and averaged 3.4% for survival of *C. dilutus*, 4.2% for growth of *C. dilutus*, 2.1% for survival of *H. azteca*, and 8.1% for growth of *H. azteca*. Overall, these quality assurance data indicate that test acceptability criteria were met, and therefore the test results can be considered valid assessments of sediment toxicity.

Sediment toxicity test results

The data for each endpoint of the sediment toxicity tests are summarized as mean values herein and are reported as the individual laboratory replicates in George et al. (2016). Survival of *C. dilutus* ranged from 76% at emil-3 to 98% at control-1 and differed significantly by site, but not by type or stream (Fig. 2). Noninferiority (relative to the mean value of the three downstream reference sites) was established for sites emil-4 and emil-5 but not for emil-3. Growth of *C. dilutus* ranged from 0.77 mg at orch-3 to 1.19 mg at emil-5 and differed significantly by site, but not by type or stream. Noninferiority was established for all three AOC sites. Survival of *H. azteca* ranged from 83% at emil-1 to 99% at emil-4 and differed significantly by site but not by type or stream (Fig. 3). Noninferiority was established for all three AOC sites. Growth of *H. azteca* ranged from 0.11 mg at emil-3 to 0.20 mg at control-1 and differed significantly by site but not by type or stream. Noninferiority was established for sites emil-4 and emil-5 but not for emil-3.

The combined results of the sediment toxicity tests indicate that sediments within the AOC generally were not significantly more toxic to the survival and growth of *C. dilutus* and *H. azteca* than sediments from the downstream reference area or the other site types. The linear mixed model indicated that none of the toxicity endpoints differed significantly between the AOC and the downstream reference area, and noninferiority of two of the three AOC sites (emil-4 and emil-5) was established for each endpoint. However, the model identified site as a significant factor for all four endpoints, which indicates that sediment toxicity varied between individual sites. The survival and growth of both test species was at or near its lowest levels at emil-3, the upstream-most AOC site. It is possible that longer-duration sediment toxicity tests such as USEPA test methods 100.4 and 100.5 (USEPA, 2000) might have been more effective at identifying chronic growth effects at sites with marginally toxic sediments (Crane et al., 2005; Ingersoll et al., 2001). However, 10-day tests remain the standard for assessments of sediment toxicity (USEPA and USACE, 1998) and have been used extensively within the AOC framework (CH2MHILL, 2012; Crane et al., 2005; Hoke et al., 1993).

Macroinvertebrate community integrity and structure

The use of Oak Orchard Creek as a comparable reference stream and the validity of comparing macroinvertebrate community integrity and structure between sites were strengthened by the relatively similar grain-size distributions and TOC values. Phi units ranged from 4.24 at emil-3 to 6.81 at emil-2 and averaged 5.08 on Oak Orchard Creek and 5.42 on Eighteenmile Creek (Table 1). The percentage of TOC ranged from 0.78% at orch-3 to 4.82% at emil-1 and averaged 2.1% on Oak Orchard Creek and 3.4% on Eighteenmile Creek (Table 1). While these habitat data suggest that comparisons of macroinvertebrate communities are appropriate and valid, a low relative abundance of organisms in both streams complicated the assessment of macroinvertebrate community integrity. Despite compositing the sieved contents from four grabs into each replicate, the desired 100-organism subsample could not be achieved for many replicates even after an exhaustive sort of the detritus. Although the low relative abundance of organisms may itself be a reflection of sediment toxicity at some sites or broad (non-AOC) regional stressors, the BAP scores derived from small subsamples may underestimate the true condition of macroinvertebrate communities. Therefore, BAP scores are presented (a) as originally intended using

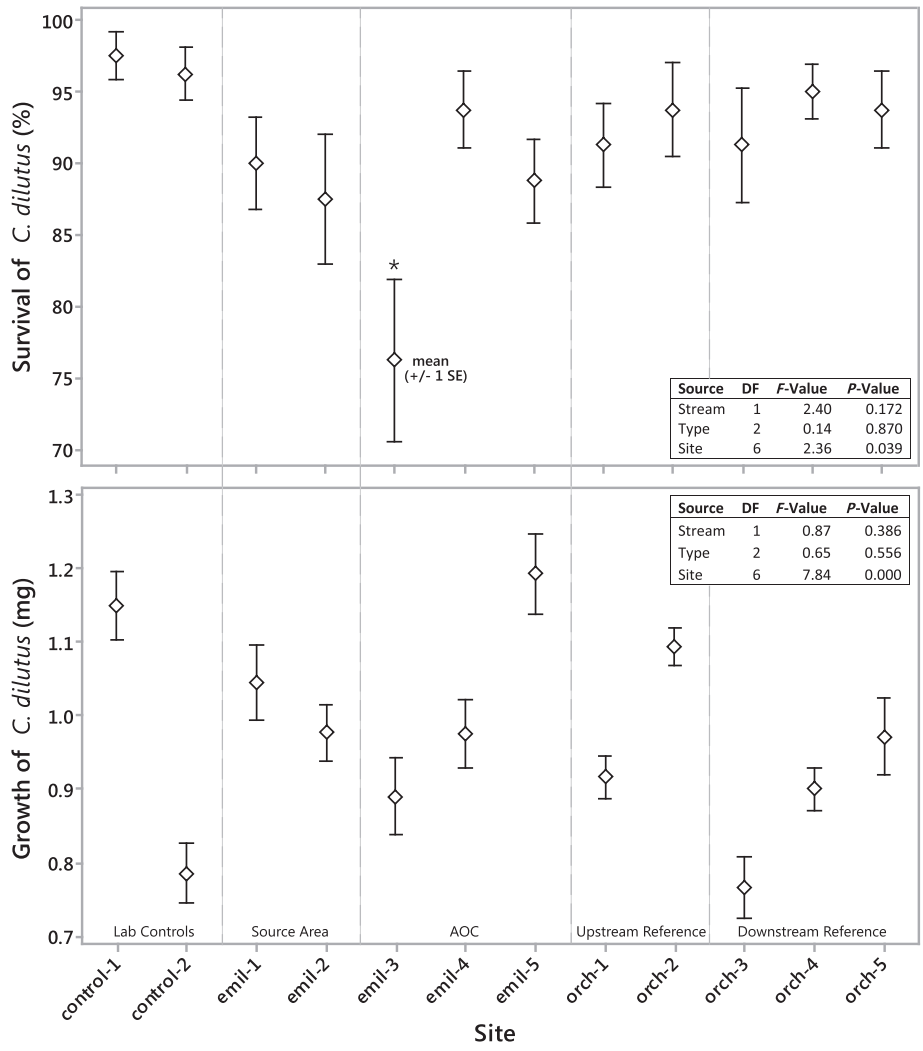


Fig. 2. Interval plots (mean ± one standard error, n = 8) showing the survival and growth of *C. dilutus* in 10-day sediment toxicity tests from ten study sites and two laboratory controls. Results of the linear mixed model show the significance of stream, type, and site on survival and growth of *C. dilutus*. Asterisk denotes an AOC site for which noninferiority could not be established with the mean downstream reference condition.

the means of the five replicates at each site (regardless of sample size) and (b) as an aggregated score (herein termed the aggregate BAP) in which the organisms from all five replicates from each site were combined, rarefied down to a random 100-organism subsample 30 times, and shown as the mean score of those 30 random subsamples. The former approach represents a consistent level of sampling effort, incorporates the density of organisms present, is appropriate for comparisons between sites, and follows standard NYSDEC protocols (Smith et al., 2014). The latter approach, by simulating the 100-organism target count, provides a community evaluation that may be more appropriate for evaluating the integrity of macroinvertebrate communities relative to the established NYSDEC impact classes and BUI removal criteria.

The integrity of macroinvertebrate communities, presented as the mean BAP score from the five replicates at each site, ranged from 2.1 at emil-3 to 5.9 at orch-2 (Table 2) and differed significantly by site but not by type or stream (Fig. 4). Similar to the results of the sediment toxicity tests, differences between sites were highly significant, and BAP score was lowest at emil-3. Noninferiority was established for site emil-5 but not for emil-3 and emil-4. For biological monitoring of surface waters in New York State, the BAP score is interpreted on a four-tiered scale of water quality impact ranging from severely impacted to non-impacted (Smith et al., 2014). The aggregate BAP scores ranged from 3.9 at emil-3 to 7.5 at emil-5, and emil-2 and emil-3 were classified as moderately impacted, emil-1, emil-4, orch-1, orch-2, orch-3, orch-4 and orch-5 were classified as slightly impacted, and emil-5 was classified as non-impacted (Fig. 4). Together, the results of the mean BAP scores and the aggregate BAP scores indicate that community integrity was poorest immediately upstream and downstream of Burt Dam and that macroinvertebrate communities across most sites on both streams showed some degree of departure from the expected unimpacted condition.

Multivariate analysis of the macroinvertebrate assemblages indicated that community structure differed between the four site types. The ANOSIM test indicated that type was a significant factor (Global $R = 0.430$, $P = 0.001$) and pairwise comparisons were significant between all site types (Fig. 5). However, the relatively small *R*-values indicate that differences between site types, while significant, were minimal and should be interpreted cautiously. It is noteworthy that the AOC sites on Eighteenmile Creek were actually more similar to the downstream reference sites on Oak Orchard Creek than to the source area sites on Eighteenmile Creek, which are located <2.5 km upstream of the AOC. The nMDS ordination showed that two of the AOC sites, emil-4 and emil-5, grouped closely with the three downstream reference sites, while emil-3 separated from these five sites (Fig. 5). The SIMPER analysis indicated that the most discriminating taxa between emil-3 and the three downstream reference sites were three chironomid genera, *Chironomus* sp., *Microchironomus* sp., and *Procladius* sp., and one oligochaete species, *Limnodrilus hoffmeisteri*, which

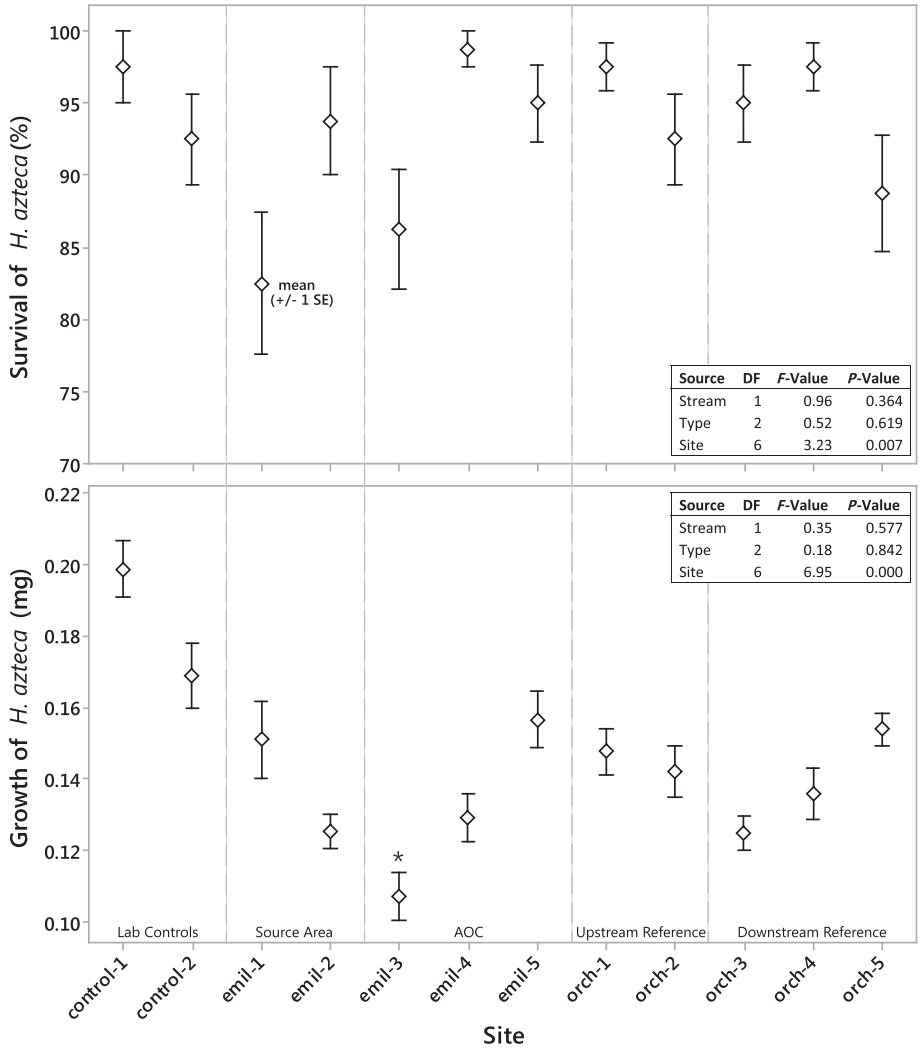


Fig. 3. Interval plots (mean ± one standard error, n = 8) showing the survival and growth of *H. azteca* in 10-day sediment toxicity tests from ten study sites and two laboratory controls. Results of the linear mixed model show the significance of stream, type, and site on survival and growth of *H. azteca*. Asterisk denotes an AOC site for which noninferiority could not be established with the mean downstream reference condition.

together contributed 30.8% of the overall dissimilarity. *Chironomus* sp. and *Microchironomus* sp. were completely absent from emil-3 while *L. hoffmeisteri* was present at emil-3 but in low abundances. In contrast, *Procladius* sp. had a greater mean abundance at emil-3 than at the downstream reference sites and was the most abundant taxon in four of the five replicates from emil-3. The genus *Procladius* is known to be extremely tolerant of environmental contamination (Warwick, 1989), and its dominance at emil-3 may be further evidence of sediment toxicity at this site.

Conclusions

The results of the sediment toxicity tests and analyses of macroinvertebrate community integrity and structure consistently supported

Table 2
Macroinvertebrate community information including the mean values for subsample size, component metrics of the Biological Assessment Profile (BAP) score, and the final (10-scaled) BAP score for the five replicates collected from each site. The aggregate BAP score presents the mean score of 30 random 100-organism subsamples from the combined replicates at each site.

Site ID	Subsample size (no. organisms)	Species richness	Hilsenhoff Biotic Index	Shannon-Weiner diversity	Percent model affinity	Dominant-3	BAP score	Aggregate BAP score
emil-1	65	11.0	8.6	2.7	60.5	68.4	4.9	5.2
emil-2	31	6.6	9.0	1.9	46.3	83.6	2.4	4.9
emil-3	15	5.4	8.6	1.8	24.6	82.0	2.1	3.9
emil-4	26	9.4	8.7	2.6	42.4	68.6	3.9	6.4
emil-5	19	10.6	8.0	3.1	42.3	51.7	5.4	7.5
orch-1	32	12.6	8.0	3.2	37.6	52.4	5.4	7.2
orch-2	81	15.2	8.4	3.2	48.6	55.3	5.9	6.6
orch-3	25	10.4	8.2	3.0	34.7	56.8	4.8	6.1
orch-4	40	11.4	8.5	3.0	36.2	56.3	4.8	7.1
orch-5	25	9.4	8.8	2.6	48.8	64.2	4.3	6.0

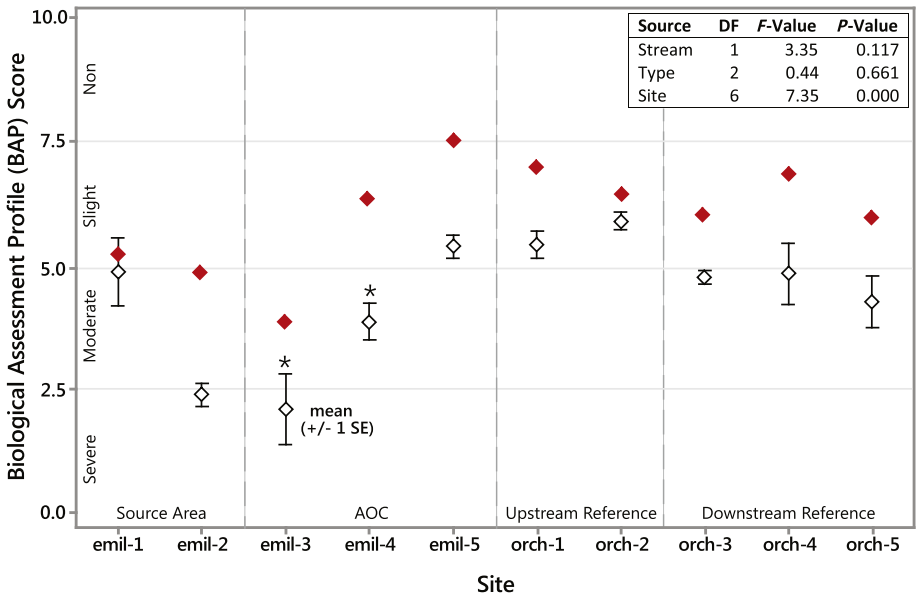


Fig. 4. Biological Assessment Profile (BAP) scores of macroinvertebrate community integrity from ten study sites shown as hollow diamonds for the mean values (±one standard error, n = 5) and as solid diamonds for the aggregate BAP values. Results of the linear mixed model show the significance of stream, type, and site on the BAP score. Asterisk denotes an AOC site for which noninferiority could not be established with the mean downstream reference condition.

two important conclusions. First, both analyses indicated that the overall quality of bed sediments in the AOC was not significantly worse than that of the downstream reference sites on Oak Orchard Creek. This finding was supported by the results of four endpoints from two toxicity tests and by a multi-metric index of macroinvertebrate community integrity. The only analysis to find a significant difference between AOC and downstream reference sites was a multivariate analysis of macroinvertebrate community structure using an ANOSIM test. This difference was relatively small and does not necessarily imply that conditions were worse in the AOC, only that communities were slightly different. Second, although our results indicated that the overall quality of sediments in the AOC was no worse than at a comparable reference stream, one site within the AOC, emil-3, consistently scored poorly in all analyses. Sediments from emil-3 had the lowest or among the lowest survival and growth of both test species in the toxicity tests, the lowest BAP scores of macroinvertebrate community integrity, the lowest density of organisms, and emil-3 was somewhat isolated in the nMDS ordination of macroinvertebrate community structure. Additionally, noninferiority could not be established at emil-3 for survival of *C. dilutus*, growth of *H. azteca*, and BAP score, relative to the respective mean values of the three downstream reference sites. These results suggest that sediment toxicity may be adversely affecting macroinvertebrate communities at emil-3 which is the upstream-most AOC site, located approximately 1.2 km downstream from Burt Dam. This is somewhat consistent with findings by Pickard (2006) indicating that the concentrations and bioavailability of most toxic substances were generally greater in the upper 2 km of the AOC. However, the nearest individual sampling point from that study, located approximately 40 m from emil-3, was not among the most contaminated of the 15 sites examined (Karn et al., 2004; Pickard, 2006). This may reflect the patchy nature of

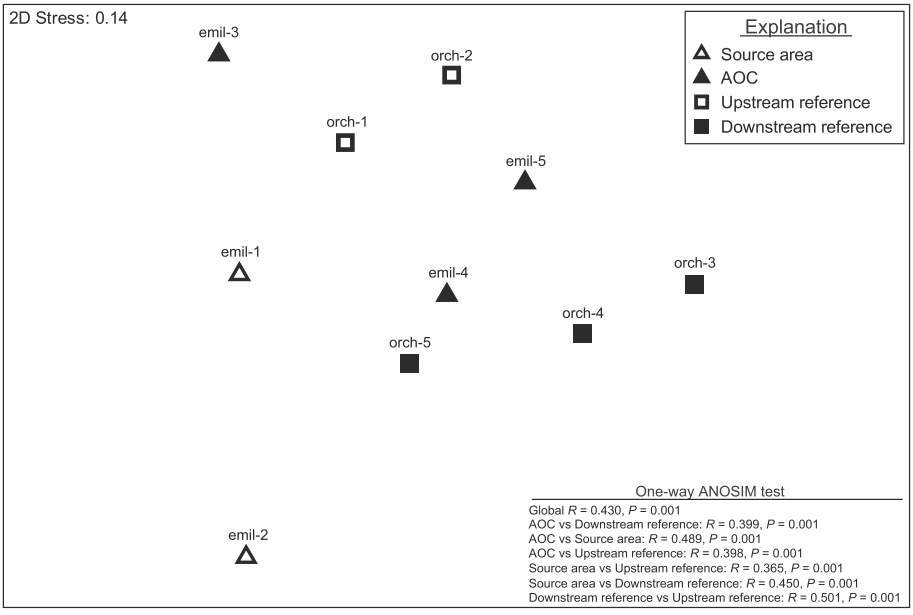


Fig. 5. Non-metric multidimensional scaling ordination and analysis of similarities (ANOSIM) results comparing macroinvertebrate community structure between site types. The ANOSIM test was run using each replicate from each site while the ordination shows the combined assemblage (sum of all replicates) from each site.

sediment contamination, particularly in lotic environments (Batley et al., 2002; Burton and Johnston, 2010; Crane and MacDonald, 2003), and underscores the importance of extensively evaluating sediments prior to remedial efforts (Batley et al., 2002). Together, the results from this study coupled with those of Pickard (2006) may identify emil-3 and the upper 2 km as a potentially impacted reach that could be targeted for more intensive sampling or future remedial efforts. Two recent studies, however, found that the concentrations of many toxic substances including PCBs and metals were at least 10–20 times higher in the source area upstream of Burt Dam (CH2MHILL et al., 2015; Stackelberg and Gustavson, 2012) than in the AOC. Given this, it is somewhat surprising that similar or more severe toxicity was not apparent in sediments from the source area at sites emil-1 and emil-2.

The results of the present study have important implications for assessing the current status of the benthos BUI in the Eighteenmile Creek AOC. The general delisting guidelines from the International Joint Commission state that the benthos BUI may be removed from an AOC when benthic community structure or sediment toxicity do not differ from comparable unimpacted reference sites (IJC, 1991; NCSWCD, 2011). These or similarly structured criteria that assess difference from comparable reference conditions (Grapentine, 2009) have been used effectively to evaluate, or justify removal of, BUIs in other AOCs, including the degradation of benthos and plankton BUIs in the St. Lawrence River at Massena (Baldigo et al., 2012; Duffy et al., 2016) and Rochester Embayment (Baldigo et al., 2016) AOCs, and the fish tumors BUI in the Presque Isle Bay AOC (Rutter, 2010). The absence of notable significant differences in sediment toxicity and macroinvertebrate communities between the Eighteenmile Creek AOC and the downstream reference area in Oak Orchard Creek suggests that the quality of bed sediments inside and outside the AOC are not dissimilar. Thus, if broadly applied, the IJC BUI-removal guideline might support the removal of the benthos BUI in this AOC. Such a recommendation, however, would be complicated by the apparent sediment toxicity observed at one AOC site, emil-3. The Remedial Action Plan for the Eighteenmile Creek AOC also provides specific removal criteria for the benthos BUI, most notably that benthic macroinvertebrate communities be classified as non-impacted or slightly impacted according to NYSDEC macroinvertebrate indices (NCSWCD, 2011). The aggregate BAP scores at the three AOC sites on Eighteenmile Creek indicated that emil-3 was moderately impacted, emil-4 was slightly impacted, and emil-5 was non-impacted, thus narrowly failing to meet the specific removal criteria. However, BAP scores from the reference sites on Oak Orchard Creek also showed some degree of impact which suggests that confounding regional factors such as eutrophication or sedimentation may also be contributing to lower BAP scores. Together, the combined results from the sediment toxicity tests and assessments of macroinvertebrate community integrity and structure suggest that AOC and reference conditions are not dissimilar but some localized effects of sediment toxicity may exist.

Acknowledgements

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Appendix 3 - Mink Dietary Model

Final Project Report

for

Eighteenmile Creek Area of Concern Mink Prey Survey and Oak Orchard Creek Add-on

Project GL-00E02088-1

December 1, 2021

Revised July 21, 2022

Prepared for

Niagara County Soil & Water Conservation District
Lockport, NY

Prepared by

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Executive Summary

1. In the Great Lakes Basin, the International Joint Commission has identified 43 Areas of Concern (AOC) where pollution from past industrial production and waste disposal practices has created hazardous waste sites or contaminated sediments. Beneficial Use Impairments (BUI) have been identified for each AOC, and for an AOC to be delisted removal of each of its BUIs must be documented.
2. The American Mink (*Neovison vison*) is the most sensitive mammal in North America to polychlorinated biphenyls (PCB), dioxins (CDD) and furans (CDF). The purpose of this project was to document whether mink consuming a diet with high proportions of aquatic prey from the Eighteenmile Creek (EMC) AOC and Oak Orchard Creek (OOC) reference area (REF) would accumulate concentrations of chemicals of concern (COC) high enough to cause chronic (health) or acute (lethal) effects in mink.
3. Mink prey tissues (amphibian, crayfish, lower [LF] and upper [UF] trophic level fish, e.g., sunfish and bass, respectively) were analyzed for total mercury (THg), total PCB and co-planar PCB, CDD and CDF, and our results compared to previous studies by Brockport (Genesee River [GR] portion of the Rochester Embayment [RE] AOC, Buffalo River [BR] AOC) and Ecology and Environment, Inc. (EMC AOC, OOC REF).
4. We also used literature-based diet (using COC concentrations in composited mink prey tissue) and bioaccumulation (using COC concentrations in water) models to predict COC concentrations in mink living in the EMC AOC and OOC REF. The two models agreed within 2.6 ±1.6 pg/g PCB TEQ for EMC, and 3.2 ± 0.6 pg/g for the OOC REF. These differences as a percent of the diet model results were 4.3 ± 3.3 % for EMC, and 94.0 ± 1.0 % for OOC REF. The percent difference between the two models was much higher in the OOC REF due to the much lower PCB TEQ concentrations there.
5. TPCB and PCB TEQ concentrations in mink prey in the EMC AOC are an order of magnitude or higher than they are in the OOC REF, GR portion of the RE AOC and BR AOC. Concentrations of THg and CDD/CDF TEQ are not of biological concern to mink in any of the four locations.
6. Removal of BUIs for the EMC AOC
    1. Based on the results of this study, BUI #3, Criterion 3, *"PCB concentrations in fish tissue are below thresholds likely to result in **acute** toxicity to fish or piscivorous wildlife (birds and **mammals**)"* can be removed for the EMC AOC. Although the concentration of TPCB in the UF prey group in the AOC exceeds the acute Lowest Observed Adverse Effect Level (LOAEC) when considered in isolation, weight-of-evidence indicates that PCBs in the AOC are not likely to cause acute toxicity in mink.

2. Based on the results of this study, BUI #5, Criteria 1 "*PCB concentrations in fish tissue from comparable functional feeding groups are similar to reference sites*" OR 2 "*PCB concentrations in fish or other prey are below tissue concentrations known to cause deformities or reproductive impairment in piscivorous wildlife*" are not recommended for removal. Concentrations of TPCB and PCB TEQ are significantly higher in the EMC AOC than the GR portion of the RE AOC, BR AOC and OOC REF (Criterion 1), and concentrations of TPCB and PCB TEQ in the AOC greatly exceed their chronic LOAECs (Criterion 2).

7. Other recommendations

   a. Getting PCB TEQ, not TPCB, below chronic LOAECs in contaminated ecosystems is the best way to protect the health of piscivorous birds and mammals. In the future, the RAP Coordinating Committee should consider using water sampling and the bioaccumulation model used in this study that was optimized for the EMC AOC to predict PCB TEQ concentrations in mink. Modeled PCB TEQ concentrations ranged by factors of 4.0 to 9.2 higher than the 9.2 pg/g chronic LOAEC relevant to BUI #5 Removal Criterion 2. Given currently high TEQ in mink prey it will take many years for predicted PCB TEQ to fall below the LOAEC, at which time another mink prey study should be conducted so that then existing COC concentrations in prey can be used in this study's diet model. If the bioaccumulation and diet models agree that PCB TEQ concentrations are less than the chronic LOAEC, BUI #5 Removal Criterion 2 would be satisfied. An alternative to the approach described above would be to locate and remediate source areas in EMC to reduce PCB concentrations in water and, subsequently, mink prey in the AOC below chronic LOAECs for mink, which would be a long and costly process.

   b. Another approach for the RAP Coordinating Committee to consider now would be to examine the findings reported in the mink habitat suitability and signs portion of this study that led the project team to decide that a mink prey study was the only way to address BUIs #3 and #5. Mink habitat suitability was low, only one definitive mink sign was observed, and the area of the AOC is so small that only 1-2 male mink at a time could hold territories there. While some mink may pass through the AOC to reach other habitats, the AOC itself cannot sustain a viable mink population and the same is true for this study's "source area" between Ide Road and Burt Dam. While any mink living long-term in the AOC would exceed the chronic LOAEC for PCB TEQ, the RAP Coordinating Committee might consider removing BUI #5, Criterion 2 on the basis that few or no mink can be long-term residents of the AOC due to habitat quality and area constraints.

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Introduction

In the Great Lakes Basin, the International Joint Commission (IJC) has identified 43 Areas of Concern (AOC) where pollution from past industrial production and waste disposal practices has created hazardous waste sites or contaminated sediments. Beneficial Use Impairments (BUI) have been identified for each AOC, and for an AOC to be delisted removal of each of its BUIs must be documented. This study assessed whether chemicals of concern (COC) could negatively impact mink populations along Eighteenmile Creek (EMC) and addressed two BUIs: *Degradation of Fish and Wildlife Populations* and *Bird or Animal Deformities or Reproductive Problems*. Criteria for removing these two BUIs in the EMC AOC are in Table 1 and definitions of acronyms used in this report are in Table 2.

EMC AOC study area

EMC and its watershed (Figure 1) are located within Niagara County, NY, approximately 18 miles east of the Niagara River. It has three major tributaries, Gulf Creek, East Branch Creek and the New York Barge (Erie) Canal, and flows north into Lake Ontario at Olcott, NY. The AOC boundary includes Olcott Harbor and extends to the farthest point at which backwater conditions exist in EMC during Lake Ontario's highest monthly average lake level. This point is located just downstream from Burt Dam, ~2 miles south of Lake Ontario. The "Creek Corridor" in the City of Lockport, NY, and the watershed south of Burt Dam are considered contaminant "source areas" (SA). This project focused on two sections of EMC: the AOC from Lake Ontario to Burt Dam and the SA between Burt Dam and Ide Road near Newfane, NY (Figure 1).

Basis for the decision to focus on mink prey

The American Mink (*Neovison vison*) is an excellent sentinel species to use in relation to BUIs 3 and 5 for the EMC AOC (Table 1) because it is highly sensitive to COCs in the environment. This is primarily due to the high trophic level (TL) of mink, and because when living in contaminated riparian areas they consume mostly aquatic animals (cf. Alexander 1977, as cited by USEPA 1993) that often contain high concentrations of COCs. Previous research has shown that mink populations are especially sensitive to dioxins (CDD), furans (CDF) and dioxin-like coplanar polychlorinated biphenyls (PCB), which at part per billion (ng/g, total PCB) or trillion (pg/g; CDD, CDF and coplanar PCB toxic equivalent [TEQ]) concentrations cause reproductive failure. Minks are especially well suited for the EMC AOC and SA study because the concentrations of total PCB and PCB TEQ are very high in EMC. Above a whole-body residue of 9.2 pg/g, these chemicals also may cause cancerous jaw lesions, the most sensitive biomarker of effect (Haynes *et al.* 2009) known for mink. Studies in the 1970s and 1980s showed that organo-chlorine pesticides failed to present significant toxicological effects for mink (Giesy *et al.* 1994). They would be even less suitable for study now because concentrations of these chemicals in the environment have decreased.

In August 2018, all muddy areas along the entire EMC shoreline between Lake Ontario and the southern extent of the Burt Dam reservoir were examined for mink footprints by an

experienced trapper and the field crew (Figure 2). Also, logs and rocks along shore were checked for mink scat and other signs (Lesmeister and Nielsen 2011, Yamaguchi and Macdonald 2003, Birks and Linn 1982). From Lake Ontario to Burt Dam, one definitive (several distinct tracks) and three faint, potential signs of mink were observed. No signs of mink were observed along the shoreline of the SA (Haynes and Wellman 2019).

In August 2018, the experienced mink trapper and field crew made observations, by boat and on foot, of potential mink habitat along the entire EMC shoreline between Lake Ontario and the southern extent of Burt Dam reservoir (Figure 2). Following the Riverine-Lacustrine Suitability Index (SIRL, Allen 1986) and the trapper's experience, mink habitat suitability scores were calculated for each of the 36 stream reaches surveyed. Both the SIRL (mean = 31%) and the trapper's (mean = 32%) scores rated mink habitat quality low in the entire study area (Haynes and Wellman 2019). Based on these results (Figure 2) and the small size of the study area, the EMC project team decided that the study area would not contain enough mink to achieve project objectives (at least 20mink needed to be trapped). Accordingly, as anticipated in the QAPP, we switched to a mink prey study that would allow us to estimate exposures of mink to total mercury (THg), PCBs and CDD/CDFs. In 2020, the project was expanded to include selected sampling in Oak Orchard Creek (OOC), which is a long-time reference area (REF) for AOC and Superfund studies, so that we could construct diet and bioaccumulation models for mink exposure to COCs in the OOC REF.

Study objectives and hypotheses

EMC AOC and SA

Objective 1: Collect potential aquatic prey species of mink and analyze them for COCs to determine whether the health of mink would be at risk if they consumed prey living in the AOC and SA. Prey tissues were analyzed for CDD/CDF and PCB congeners and THg. Potential terrestrial prey species were not collected because they are primarily herbivores that contribute very low contaminant concentrations to a mink's diet compared to aquatic prey.

Objective 2: Use COC concentrations in the available prey for EMC mink to construct a diet model to estimate consumption risks for mink, then compare model predictions and analytically determined concentrations in sampled prey to published dietary lowest observed adverse effect concentrations (chronic LOAECs).

Objective 3: Compare COC concentrations determined for mink prey in the AOC and SA to mink prey in other AOCs (Buffalo River AOC, BR AOC; Genesee River portion of the Rochester Embayment AOC, GR AOC, and the OOC REF area between the Waterport Station Dam and Lake Ontario (Figure 3).

Objective 4: Collect whole water samples (dissolved and particulate fractions) from the EMC AOC and SA and in Lake Ontario (LO) away from tributary influences in spring, summer and fall seasons. Whole water samples also were collected in the OOC REF by

the U.S. Army Corps of Engineers (USACE) in March 2021 and August 2020 and by the U.S. Environmental Protection Agency from 2005-2010 (Data provided by Andrew Lennox, USACE Buffalo District). COC concentrations in whole water samples were the foundation for bioaccumulation model used to predict chemical concentrations in mink diets in EMC AOC and SA and OOC REF.

Hypothesis 1: COC concentrations in mink prey do not differ significantly between the EMC AOC and SA.

Hypothesis 2: COC concentrations in mink prey do not differ significantly among the EMC AOC and SA, BR AOC, GR portion of RE AOC and OOC REF.

Hypothesis 3: COC concentrations in mink prey in the EMC AOC and SA, BR AOC, GR portion of RE AOC and OOC REF are not higher than published dietary LOAECs.

Hypothesis 4: COC concentrations in water from EMC AOC and SA, OOC REF and LO away from tributary influences are not significantly different.

Diet model

Objective 5: Use literature reports of mink diets (USEPA 1993) and trophic levels of their prey to construct both worst case (92% aquatic with 58% high trophic level fish, thus highest potential exposure to contaminants) and likely case (65% aquatic and 35% terrestrial) diet models, including literature-based proportions of four aquatic prey groups: amphibians (AM), crayfish (CR), and lower (LF) and upper (UF) trophic level fish.

Objective 6: Use diet models to estimate exposure of mink to COCs in the EMC AOC and SA and compare diet modeling results to published LOAECs.

Hypothesis 5: COC concentrations estimated by diet models using data for the EMC AOC and SA are not significantly different.

Hypothesis 6: COC concentrations estimated by diet models using data for the EMC AOC and SA are not higher than published dietary LOAECs.

Oak Orchard Creek reference area

OOC (Figure 3) is a reference water body (no known sources of COCs beyond background levels) used by federal and state agencies to compare with chemical and biological findings from AOC and Superfund studies. Brockport's sampling for the OOC REF included collection and analysis of COCs in CR.

Objective 7: Use COC data from a previous study (E & E 2019) for THg and PCB (total and TEQ) in LF and UF common to EMC and OOC, along with crayfish COC data collected by Brockport, to model mink diet in EMC and OOC and compare it to published LOAECs.

Hypothesis 7A: COC dietary exposure estimates for the OOC REF are not significantly different from dietary exposure estimates for EMC SA and AOC.

Hypothesis 7B: COC concentrations estimated by diet models using data for the OOC REF are not higher than published dietary LOAECs.

Bioaccumulation model

Objective 8: Modify a published bioaccumulation model (derived from Sample *et al.* 1996 by Wellman *et al.* 2009 and Wellman 2006) to reflect concentrations of PCB TEQ measured in EMC water. The resulting model will allow prediction of COC concentrations in mink diet based on concentrations in whole water, and comparison of those predicted concentrations with diet models and published LOAECs.

Objective 9: Use the bioaccumulation model developed for EMC (Objective 8) to estimate COC concentrations in mink diet from concentrations in whole water collected from the OOC REF.

Hypotheses 8-9: Predictions of the bioaccumulation models for the EMC and OOC REF will match (±20%) predictions of the EMC and OOC REF diet models.

BUI removal criteria

Objective 10: Evaluate BUI 3, *Degradation of Fish and Wildlife Populations*, removal Criterion 3, "PCB concentrations in fish tissue and other prey are below thresholds likely to result in **acute** toxicity to fish or piscivorous wildlife (birds and **mammals**)."

Objective 11: Evaluate BUI 5, *Bird or Animal Deformities or Reproductive Problems*, removal Criterion 1, "PCB concentrations in fish tissue from comparable functional feeding groups are similar to reference sites" **OR** Criterion 2, "PCB concentrations in fish or other prey are below tissue concentrations known to cause deformities or reproductive impairment in piscivorous wildlife". This study evaluated both criteria.

Hypothesis 10: PCB concentrations in fish tissue and other prey are below thresholds likely to result in acute toxicity to fish or piscivorous mammals.

Hypothesis 11A: PCB concentrations in fish tissue from comparable functional feeding groups are similar to reference sites.

Hypothesis 11B: PCB concentrations in fish or other prey are below tissue concentrations known to cause deformities or reproductive impairment in piscivorous wildlife.

Use of Toxic Equivalency Factors and Toxic Equivalents for PCBs

Observed toxic effects of PCBs are predominantly caused by interaction of coplanar PCBs (and also co-planar CDDs and CDFs) with the aryl hydrocarbon receptor (AhR, Giesy and Kannan 2002, Van den Berg *et al.* 2006) and not TPCB concentration, *per se*. Toxic effects of PCB congeners interacting with the AhR can be described by toxic equivalency factors (TEF) that quantify the relative toxic effects of co-planar COCs in terms of the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, TEF = 1), the most toxic co-planar COC (Van den Berg *et al.* 2006). Ortho-substituted (non-coplanar) PCBs do have adverse effects (e.g.,

neurological, hormonal), but only at very high concentrations, so they are not likely to significantly contribute to toxic effects at ecological concentrations (Giesy and Kannan 2002). Environmental weathering (including bioaccumulation) of PCBs increases proportions of coplanar PCBs in mixtures, thus weathered PCB mixtures are more toxic than their parent technical mixtures (Giesy and Kannan 2002). Because it accounts for these weathering effects and for toxicity of PCB congeners at ecological concentrations, TEQ provides a better indicator of hazard to wildlife than do TPCBs (Giesy and Kannan 2002). Hence, this study reports TEQ for PCBs and total TEQ, including coplanar CDDs/CDFs, and TPCBs.

Materials and Methods

Field sampling

From fall 2018 to fall 2020, three whole water samples were collected at three locations in the EMC AOC and SA and >1 mi. offshore in Lake Ontario away from tributary influences, one sample at each location in spring, summer and fall (Table 3). Water samples were collected in hexane-rinsed, labeled 3.8L brown glass bottles and placed on ice in coolers immediately. Upon return to the Brockport Lab, unfiltered water was refrigerated before next day, overnight shipment on wet ice to ALS Environmental, Kelso, WA, for chemical analyses of PCB, CDD and CDF congeners and THg (see table 2 for definitions of acronyms).

In suitable shallow habitat, 40-50 CR were caught by hand after flipping rocks in EMC and OOC. In EMC, 10 AM were caught with long-handled dip nets in suitable riparian habitat, and 10 LF and 5 UF were caught by boat electrofishing then placed in an aerated live well for later sorting and processing of fish to be kept for analysis or released alive. A minimum of 70 g of each prey type (1g for stable isotope analysis and 20g each for THg, PCB congener and CDD/CDF congener analyses) was collected in three sampling seasons (Table 3). Captured prey were placed in zip lock bags on ice in the field (one each for CR, AM, LF and UF).

Lab processing of samples

Within 24-48 h, prey organisms were processed in the Brockport Laboratory and frozen.

1. Specimens in each of four mink prey trophic groups collected during each sampling season were identified, measured (mm) (fish: tip of snout or lower jaw to tip of caudal fin; crayfish: tip of rostrum to tip of telson; amphibians: snout to vent), and weighed (g) with a digital top-loading scale.
2. With hexane-rinsed tools, ~1g of muscle tissue was excised from ten specimens in each trophic group (five specimens of UF), placed in labeled, hexane-rinsed glass vials, frozen, and saved to ship for stable isotope analysis by the Cornell Isotope Laboratory (COIL) in Ithaca, NY.
3. The remaining tissue (>>70g) from each trophic group for each season was placed in a labeled zip lock plastic bag, frozen and, at the end of each sampling season, shipped overnight on wet ice to Kelso, WA, for chemical analyses by ALS Environmental.

4. Code numbers were placed on or in each sample container as it was filled. With their code numbers, all data from field and lab data sheets were entered into spreadsheets and saved on two data storage devices (lab computer, Project Director's home computer) within 24 h.
5. Upon receipt, COIL and ALS Environmental froze and subsequently thawed, ground up and homogenized (ALS) or freeze dried (COIL) specimens from the prey trophic groups. At ALS, each of the three seasonal composited samples for each trophic group was split into four aliquots and frozen in labeled, hexane-rinsed glass. Tissue samples were analyzed by for THg, PCB congeners (the sum of which gave TPCB) and CDD/CDF congeners. The 12 coplanar PCB congeners and 17 CDD/CDF congeners with TEFs were used to calculate PCB TEQ and CDD/CDF TEQ, respectively. Excess tissue was frozen in reserve jars for contingencies.

Mink hazard assessment

Prey group samples

Concentrations of THg, TPCB and TEQ for CDD/CDF and coplanar PCB congeners found in mink prey were compared to published chronic and acute LOAECs. For THg, chronic and acute LOAECs are 500 ng/g and 1,000 ng/g, respectively (Dansereau *et al.* 1999). For TPCB they are 960 ng/g (Bursian *et al.* 2006) and 5,000 ng/g (Aulerich and Ringer 1977). TEQs for CDD/CDF and co-planar PCB congeners were calculated using World Health Organization TEF values from Van den Berg *et al.* 2006. TEQ was summed separately for CDD/CDF and PCB congeners, then the categories were summed to yield total TEQ for each prey group sample (Electronic Appendix A: Data analysis worksheet "EMC Prey"). For PCB/CDD/CDF TEQ, singly or combined, chronic and acute LOAECs are 9.2 pg/g (Bursian *et al.* 2006) and 1,000 pg/g (Hochstein *et al.* 1998), respectively.

Stable isotope analysis to determine mink prey trophic levels

Stable isotopes of nitrogen are used to evaluate trophic webs of ecosystems to give lifetime, integrated estimates of trophic level (TL) for organisms (DeNiro and Epstein 1978, Cabana and Rasmussen 1994). ^{14}N has a stable, heavier isotope (^{15}N) which occurs naturally, and the heavier and lighter isotopes are differentially absorbed and metabolized by organisms (Fry 1991). Usually, the lighter isotope is excreted preferentially, leading to enrichment of the heavier isotope in organisms relative to their environment or diet. This enrichment is measurable through mass spectrometry and is reported in parts per thousand (δ‰) relative to a standard: $\delta X = [\frac{R_{sample} - R_{standard}}{R_{standard}}] \times 10^3$, where X is ^{15}N and R is the corresponding ratio of ^{15}N/^{14}N. The standard for nitrogen is atmospheric nitrogen (Fry 1991).

Selective excretion of ^{14}N over ^{15}N by animals results in an increase of approximately 3.4‰ in the δ^{15}N at each trophic level; thus, ^{15}N analysis of animal tissue can determine the trophic level of the animal (Peterson and Fry 1987; Cabana and Rasmussen 1994). Muscle tissue

from each trophic group in each season were analyzed by the COIL in Ithaca, NY for isotopic ratios of $^{15}N/^{14}N$ (δN) to determine the TL of composite samples for each prey category collected in EMC and OOC (Electronic Appendix A: Data worksheet "EMC TL").

Diet Model

The diet model predicts the dietary exposure to COCs of mink in a study area by combining the COC concentrations in mink prey from that area, using a weighted average in proportions consistent with that of mink diets found in the literature. The concentration of each COC in a prey group is multiplied by that prey group's proportion of the diet, and the results are summed to yield the concentration in that diet. The model can be expressed as:

$$C_D = \sum_{i=1}^{n} C_i \times F_i \ ,$$

where C_D is the concentration of the COC in the diet, n is the number of prey categories, C_i is the concentration of the COC in prey category i, and F_i is the fraction of the diet consisting of prey category i (the sum of the fractions is 1.00).

For example, assume that a mink's diet consists of 20% terrestrial herbivores, 10% crayfish, 40% lower trophic level fish and 30% upper trophic level fish and that mean TPCB concentrations in its prey's tissues are 0 ng/g in herbivores, 10 ng/g in crayfish, 12 ng/g in lower trophic level fish and 15 ng/g in upper trophic level fish. The equation would be:

$$C_D = \left(0\frac{ng}{g} \times 0.2\right) + \left(10\frac{ng}{g} \times 0.1\right) + \left(12\frac{ng}{g} \times 0.4\right) + (15\frac{ng}{g} \times 0.3)$$

$$C_D = 0 + 1\frac{ng}{g} + 4.8\frac{ng}{g} + 4.5\frac{ng}{g} = 10.3\frac{ng}{g}$$

The mink's diet would contain 10.3 ng/g of TPCB, with herbivores contributing 0 ng/g TPCB, CR 1 ng/g, LF 4.8 ng/g, and UF 4.5 ng/g. This concentration can then be compared to LOAEC dietary concentrations.

USEPA (1993) reported the results of 17 studies of mink diet at 25 different locations where the portion of the diet from aquatic sources ranged from 13.4% to 92%. Lower (e.g., sunfish, perch) and upper (e.g., black bass, pike) trophic level fish are secondary and tertiary consumers which typically comprise 50% or more of riparian mink diets (USEPA 1993). Crayfish (omnivores) and frogs (secondary consumers) typically comprise 20% or less of riparian mink diets (USEPA 1993).

We averaged the results from the six most relevant diet studies (for mink living along rivers and streams) cited by USEPA (1993; studies averaged were Hamilton 1940, Korschgen 1958, Cowan and Reilly 1973, Alexander 1977a, b, and Burgess and Bider 1980). For each prey category, we averaged the proportion of that category from all six studies to get a "typical" proportion of the diet for that category. A "typical" riparian mink's diet consists of 33.3% UF, 13.5% LF, 10.2% crustaceans and 8.1% AM, with a total of 65% from aquatic sources.

The maximum potential dietary exposure of mink to COCs in EMC AOC water would be best represented by a study on a river in lower Michigan (Alexander 1977 cited by USEPA 1993), consisting of 57.5% UF, 27.5% LF, 4% crustaceans and 3% AM (total 92% aquatic), and 8% "other" (birds, mammals, vegetation and unidentified). We used these dietary percentages to represent a "worst-case" dietary exposure to mink of THg, co-planar PCB and CDD/CDF.

Comparison of preliminary calculations of the trophic level (see next section) of the "worst-case" diets in EMC with previous studies of the trophic level of mink in the Lower Great Lakes indicated that Alexander's diet (1977 cited by USEPA 1993) was not a good representation of mink in EMC. Studies in the Rochester Embayment AOC (Haynes *et al.* 2007) and Niagara River AOC (Haynes *et al.* 2016) measured the trophic levels of 63 trapped mink. Of those, one was at trophic level 5.13, one at trophic level 5.00, and the rest were below trophic level 5. Thus, we concluded that a more realistic "worst-case" mink diet in the EMC AOC would be at trophic level 4. To create a diet model for trophic level 4, we started with the diet proportions of the worst-case diet (Alexander 1977, cited by USEPA 1993) and, keeping the relative proportions of the aquatic prey categories constant, increased the percentage of terrestrial prey until the trophic level came down to 4.

Since we were not able to obtain amphibian samples from the EMC AOC, we had to adjust the proportions of the other prey groups to account for the missing category. For the worst-case diet scenario in the AOC, we wanted to maintain the 92% aquatic value, so we distributed the AM portion proportionally over the other three aquatic categories. For the typical diet in the AOC, we added the AM portion to the terrestrial portion of the diet, resulting in a 57% aquatic diet. We calculated diet models in the EMC SA both with and without AM.

Dietary exposures of mink in the EMC AOC and SA were estimated by multiplying the average concentration of each COC contaminant in each of the four aquatic prey groups by the corresponding portion of the modeled mink diets and summing the results. We did these calculations: 1) for the worst-case diet Alexander (1977, in USEPA 1993), 2) for the typical diet represented by the average of the six studies, and 3) for a trophic level 4 diet. Again, concentrations of individual PCB and CDD/CDF congeners were multiplied by their respective TEF, then summed to yield a total TEQ for each diet. Estimated dietary exposures were then compared to published LOAECs reported by Haynes *et al.* (2007; Electronic Appendix A: Data analysis worksheet "EMC Diet").

To compare EMC SA and AOC to OOCREF, we did a separate set of diet model calculations. In this case, we used data from CR caught in all three areas during this study along with data for pumpkinseed (*Lepomis gibbosus*, LF) and largemouth bass (*Micropterus salmoides*, UF) from E & E (2019), as these were the relevant species for which E & E had congener-specific PCB data in all three areas. We used the same diet concentrations as in the previous model for the amphibian-free typical diet (57% aquatic) and the worst-case diet (92% aquatic). This allowed direct comparison of the EMC SA and AOC to the OOC REF (Electronic Appendix A: Data analysis worksheet "OOC Comp").

Trophic level of diet

The mean trophic level for each aquatic prey group in EMC was multiplied by that prey group's proportion in the diet (the non-aquatic portion of each diet was assumed to be trophic level 1), and the results were summed to estimate the trophic levels of the model diets above (Electronic Appendix A: Data analysis worksheet "EMC Diet"). The estimated dietary trophic levels were then used in a hazard estimate by comparison with known trophic levels of mink (hence diet) determined in the RE AOC by Haynes *et al.* (2007) and the Niagara River AOC by Haynes *et al.* (2016). These trophic level estimates were also used to determine the trophic levels used in the bioaccumulation model.

Bioaccumulation Model

The bioaccumulation model, as described in Wellman *et al.* (2009), is based on Sample *et al.* (1996) and Van Gestel *et al.* (1985). Like the diet model, the bioaccumulation model also predicts the dietary exposure of mink to a persistent organic compound, which allows direct comparison of the two models. In contrast to the diet model, which uses concentrations of COCs in mink prey, the bioaccumulation model is based on each compound's total (i.e., dissolved plus particulate fractions) concentration in water, the log K_{ow} of the compound, and the trophic level of the diet. We adapted this model to estimate the dietary exposures of mink in the EMC areas to THg and PCB TEQ. We could not model bioaccumulation of TEQ from dioxins and furans because they are at least partially metabolized, an element for which this model cannot account.

The estimated dietary exposure, C_D, for each compound is found using this equation from Wellman *et al.* (2009), derived from Equation 28 in Sample *et al.* (1996):

$$C_D = \frac{C_w[100g + (177g \times P_{aq} \times BAF)]}{760g}$$

where C_w is the concentration of the congener in water, 100 g and 177 g are the daily water and food consumption rates by the mink, 760 g is the average mass of the mink (Wellman *et al.* 2009), and P_{aq} is the percent of the diet that is aquatic. Diets at trophic level 3.6 were assumed to be 65% aquatic in the SA with amphibians and 57% aquatic in both SA and AOC without amphibians. The terrestrial fraction required to force the diets to trophic level 4 varied between areas and due to presence or absence of amphibians; thus, the trophic level 4 diets ranged from 66.8% to 70.9% aquatic.

The Bioaccumulation Factor (BAF) for each compound at each trophic level is the product of the bioconcentration factor (BCF) and the Food Chain Multiplier (FCM). Given the log K_{ow} of each compound, the BCF can be calculated using a linear equation: log BCF = a log K_{ow} – b (Van Gestel *et al.* 1985, Sample *et al.* 1996).

Tables of FCM are found in Sample *et al.* (1996) and USEPA (2003, 2012a, and 2016). The calculations by USEPA (2003) are based on the model in Gobas (1993) describing the Lake

Ontario food web. USEPA (2016) uses the same values. These values are slightly lower than those found in Sample *et al.* (1996) and in USEPA (2012a). Since USEPA (2003) states that more pelagic-based food webs will have lower FCMs than more benthic-based webs, we used the values from Sample *et al.* (1996) and USEPA (2012a) as a better representation of the EMC ecosystem. As FCMs are provided, in all sources, for only one decimal place in the log K_{ow}, and only for integer TLs from 2 to 4, we interpolated to get the FCM for each compound, and for trophic levels between 3 and 4 (Electronic Appendix A: Data analysis worksheets "FCMs" and "H_2O BA"). Once the bioaccumulated concentration C_D was determined for each PCB congener, the TEQ for each was calculated by multiplying that concentration by the TEF (Van den Berg *et al.* 2006). Finally, the bioaccumulated TEQs were summed for all coplanar congeners to yield an estimate of TEQ from PCBs in the minks' diet. This was done for each trophic level of interest (Electronic Appendix A: Data analysis worksheet "H_2O BA").

To match the Bioaccumulation Model to the Diet Model results, we had to select values for *a* (slope) and *b* (intercept) in the linear equation: log BCF = *a* log K_{ow} + *b* (Van Gestel *et al.* 1985, Sample *et al.* 1996). We compared the TEQ from PCBs for each case (described by location and trophic level) in the bioaccumulation model to the TEQ from PCBs in the diet model for the same case. In selecting *a* and *b*, we chose to minimize the root sum of squares (RSS) of the differences between the two models' TEQs in the EMC SA and AOC for the typical diet and the (amphibian-free) TL = 4 diet. RSS was used so that differences with opposite signs would not cancel each other in the optimization measure; it also tends to keep all the differences close to the same size, thus optimizing equally for all included data points.

Van Gestel *et al.* (1985) reported the results of twelve studies done from 1974 to 1983 in which values for *a* ranged from 0.542 to 1.53 and values for *b* ranged from -3.03 to 0.7285. They concluded that the most reliable equation in their study was that from Veith and Kosian (1983, cited by Van Gestel *et al.* 1985), which used 122 chemicals with a large range of K_{ow}s. Thus, Van Gestel *et al.* (1985) recommended the values of *a* = 0.79 and *b* = -0.40.

Using an Excel macro (written by J. Wellman, 2021), we created a table showing the Root Sum of Squares (RSS) values for combinations of *a* and *b* in these ranges. This resulted in a diagonal "trench" of minima extending from *a* = 1.05, *b* = -2.9 to *a* = 0.5, *b* = 0.8. There was little meaningful difference between the local minima at either end of the table within these ranges. We decided to keep Van Gestel *et al.*'s (1985) recommended value for the slope a = 0.79 and find the value for the intercept b that would minimize the RSS and thus best match the diet model results (Electronic Appendix A: Data analysis worksheet "BA Macro").

We chose to match the EMC bioaccumulation model to the EMC diet model based on our composited samples, not including any E & E (2019) samples, because we could better represent the mink diet in EMC by including more species of fish, and we wanted to avoid any potential errors due to conversion of E & E's fillet concentrations to whole fish concentrations. We then used this bioaccumulation model, optimized for EMC using our samples, to predict PCB TEQ in OOC REF. However, for that comparison with the OOC REF bioaccumulation model,

we had only the OOC REF diet model based on E & E (2019) data that required conversion of fillet to whole fish concentrations (Skinner *et al.* 2009).

Data comparability and statistical analyses used

Statistical comparisons among locations for which we had equivalent data for this and historical (E & E 2019; Haynes and Wellman 2015 a, b) studies were made for non-lipid-adjusted concentrations of total mercury (THg), total PCB (TPCB), PCB TEQ and CDD/CDF TEQ. No lipid adjustments were made because in the wild mink consume most soft tissues of their prey (perhaps not the gall bladder). For TEQ we focused on PCBs because they alone exceeded mink dietary LOAECS in EMC, whereas CDD/CDF TEQ comprised only 5.1 ± 4.6% of total TEQ across this study and did not cause any LOAECs to be exceeded when added to PCB TEQ.

Composited samples of crayfish and lower trophic level fish (mostly pumpkinseed and bluegill) were analyzed for THg, TPCB and PCB TEQ in this and historical studies (E & E 2019; Haynes and Wellman 2015 a, b), but not upper TL fish. Brockport analyzed composited samples of upper TL fish and E & E analyzed skin-on fillets of individual upper TL fish, in both cases northern pike (*Esox lucius*) and largemouth bass. To address the difference among composited samples and individual fillets of fish, we constructed composited samples of E & E individual fillets, consisting of one pike and three bass, then calculated the mean concentration of the four fillets in each new composited "sample." Based on fillet data published by Skinner *et al.* (2009), we multiplied mean composited fillet concentrations by 2.8 (conversion factor for largemouth bass) to estimate mean whole-body concentration for each constructed composited sample. The reasonableness of this approach depends on three assumptions:

1. Weights of composited fish were similar across Brockport and E & E samples. Because we did not record and E & E (2019) did not report weights of individual UF fish, we could not apply the formula used by Skinner *et al.* (2009) to convert COC concentrations in individual fish fillets to concentrations in individual whole fish.
2. Conversion factor (2.8 for largemouth bass; Skinner *et al.* 2009) from fillet concentration to whole body is similar for northern pike (for which no conversion factor was provided by Skinner *et al*.) and smallmouth bass that were included in some Brockport UF composited samples.
3. Taking the constructed composite sample approach for statistical comparisons of COC concentrations among the five locations where UF fish have been collected by Brockport and E & E (2019) is better than comparing composited to individual fillet samples.

Because treatment data were not normally distributed with equal variance, non-parametric statistics were used. The Wilcoxon Rank Sum Test (WRS) was used for two-sample tests and Kruskal-Wallis AOV of Ranks (KW) was used for three or more sample tests ($\alpha = 0.05$) of null hypotheses (Statistix 2013). When a KW test was significant, Dunn's All Pairwise Comparison test (DAPC) was used to distinguish significant differences among treatments. All statistical analysis results are in Electronic Appendix B.

Results

Species composition and trophic levels of potential mink prey collected in this study

One species of CR, the northern clearwater crayfish (*Orconectes propinquus*), was collected in the EMC SA and AOC and in the OOC REF. No AM, LF and UF were collected during this study in the OOC REF. Three AM species were collected in the EMC SA: green frog, (*Lithobates* [formerly *Rana*] *clamitans*), leopard frog, (*L. pipiens*) and American toad (*Anaxyrus americana*). Only two American toads were observed in the EMC AOC, so no chemical data for amphibians could be obtained. LF species included in each composited sample were mostly bluegill (*Lepomis macrochirus*), pumpkinseed and a few yellow perch (*Perca flavescens*), UF in each composited sample was mostly largemouth and smallmouth (*Micropterus dolomieu*) bass and one northern pike (Table 4; see Table 2 for acronym definitions).

In the EMC AOC, TL (standard deviation), was 3.98 (0.06) for CR, 4.82 (0.21), for LF and 5.14 (0.27) UF) (Table 5). In the EMC SA, trophic level was 2.50 (0.13) for AM), 3.80 (0.21) for CR, 4.43 (0.11) for LF, and 5.20 (0.06) for UF (Table 5). Only crayfish were collected in OOC, and their trophic level was 4.37 (0.06). When AOC and SA TLs were averaged, there were statistically significant differences in trophic levels of the four mink prey groups (KW: $p < 0.0001$; DAPC: UF > CR & AM; LF = AM, CR & UL) (Electronic Appendix B, worksheet "Brkprt Mink Prey Results").

BUI contaminant concentrations in the tissue of likely mink prey in EMC AOC and SA and OOC REF

Amphibian tissue did not exceed dietary LOAECs for mink for any COC (Table 5). See Electronic Appendix B, worksheet "Brkprt Mink Prey Results" for the statistical data and calculations that provided the results reported in this section.

Total Mercury

For CR, concentrations of THg did not differ significantly among the EMC AOC (35.8 [38.3] ng/g), SA (30.2 [26.0] ng/g), and OOC REF (13.5 [2.7] ng/g) (KW: $p = 0.6262$). Brockport did not collect fish in OOC. For LF (WRS: $p = 1.000$) and UF (WRS: $p = 1.000$), THg also did not differ significantly between EMC AOC and SA (Table 5). CR, LF and UF did not exceed the acute (1,000 ng/g) or chronic (500 ng/g) dietary LOAEC for THg (Dansereau *et al.* 1999). UF, with the highest concentration of THg, was less than half the chronic LOAEC (Electronic Appendix A, worksheet "EMC Prey").

Total PCB

Concentrations of TPCB for CR in the SA (1,210 [1,413] ng/g) exceeded concentrations in the OOC REF (7.7 [3.7] ng/g) but not in the AOC (293 [170] ng/g) (KW: 0.0067; DAPC: EMC SA > OOC REF; EMC AOC = EMC SA & OOC REF). TPCB in CR in the SA exceeded the chronic LOAEC (960 ng/g; Bursian *et al.* 2006), but not in the AOC. Despite the large difference in mean TPCB, the concentrations in LF in the SA (2,307 [950] ng/g) were not statistically greater than in the AOC (619 [534] ng/g) (WRS: $p = 0.2$). The concentration of TPCB in LF was greater in the EMC SA than the chronic LOAEC (960 ng/g) but less than the acute LOAEC of 5,000 ng/g (Aulerich and Ringer

1977). For UF there was no statistically significant difference in TPCB concentrations between the SA (3,830 [468] pg/g) and AOC (6,395 [4,977] pg/g) (WRS: p = 0.7). Tissue concentrations of UF in the SA were greater than the acute LOAEC (5,000 ng/g), but not in the AOC, for TPCB. (Table 5; Electronic Appendix A, worksheet "EMC Prey").

PCB TEQ

Concentrations of PCB TEQ for CR in the SA (10.4 [8.6] pg/g) exceeded concentrations in the OOC REF (0.3 [0.02] pg/g) but not in the AOC (5.2 [6.7] pg/g) (KW: 0.0155; DAPC: SA > OOC REF, AOC = SA & OOC REF). CR PCB TEQ concentration in the SA was slightly higher than the chronic LOAEC (9.2 pg/g, Bursian *et al.* 2006). For LF, the PCB TEQ concentration in the SA (57 [44] pg/g) was not significantly greater than in the AOC (12 [16] pg/g) (KW: 0.2; AOC = SA), but the concentrations in both the SA and AOC were greater than the chronic LOAEC. For UF, there was no significant difference in PCB TEQ between the SA (165 [118] pg/g) and the AOC (137 [159] pg/g) (WRS: p = 1.0; AOC = SA), and both were much higher than the chronic LOAEC. None of the prey group samples exceeded the acute LOAEC for PCB TEQ (1,000 pg/g; Hochstein *et al.* 1998). (Table 5; Electronic Appendix A, worksheet "EMC Prey"). PCB #126, the congener with the highest TEF (0.1), was responsible for 86.1% of the TEQ across all samples (AM, CR, LF, UF; Appendix C2).

CDD/CDF TEQ

For CR concentrations of CDD/CDF TEQ were all $\leq$ 0.7 pg/g and did not differ significantly among the AOC, SA, and OOC REF (KW: p = 0.7). For LF, CDD/CDF TEQ in the SA (1.8 [2.0] pg/g) also did not differ significantly from the AOC (0.7 [0.6] pg/g) (WRS: p = 0.1). The same was true in the SA (2.3 [0.6] pg/g and in the AOC (3.4 [2.1] pg/g) for UF. Concentrations of CDD/CDF TEQ for AM, CR, LF and UF were well below the chronic dietary LOAEC for CDD/CDF TEQ (9.2 pg/g, Bursian *et al.* 2006; Table 5; Electronic Appendix A, worksheet "EMC Prey").

Total TEQ

Total TEQ was calculated by summing PCB and CDD/CDF TEQ. Concentrations of TTEQ for CR in the SA (11.0 [8.3] pg/g) exceeded concentrations in the OOC REF (0.4 [0.06] pg/g) but not in the AOC (5.9 [6.8] pg/g) (KW: 0.0155; DAPC: SA > OOC REF, AOC = SA & OOC REF). CR PCB TEQ concentration in the SA was slightly higher than the chronic LOAEC (9.2 pg/g, Bursian *et al.* 2006). For LF, TTEQ concentration in the SA (59 [44] pg/g) was not significantly greater than in the AOC (12.9 [16.0] pg/g) (KW: 0.2), but the concentrations in both the SA and AOC were greater than the chronic LOAEC. For UF, there was no significant difference in TTEQ between the SA (165 [118] pg/g) and the AOC (137 [159] pg/g) (WRS: p = 1.0), and both were much higher than the chronic LOAEC. None of the prey group samples exceeded the acute LOAEC for TTEQ (1,000 pg/g; Hochstein *et al.* 1998); Table 5). PCB TEQ accounted for 91.8 % of TTEQ (Appendix C2; Electronic Appendix B, worksheet "Brkprt Mink Prey Results"), and adding CDD/CDF TEQ to PCB TEQ did not cause further exceedances of the chronic dietary PCB/CDD/CDF TEQ LOAEC (Table 5; Electronic Appendix A, worksheet "EMC Prey").

Comparing Brockport (2013-14, 2018-20) and E & E (2019) results

Brockport collected COC concentration data (THg, TPCB, PCB TEQ, CDD/CDF TEQ; Haynes and Wellman 2015 a, b and this study) from mink prey (AM, CR, LF, UF). Ecology & Environment (E & E 2019) also collected COC concentration data (THg, TPCB and PCB TEQ) from mink prey (CR, LF and UF but not AM). Results from Brockport studies of mink prey in the EMC AOC and SA, the OOC REF (CR only), the Genesee River (GR) portion of the Rochester Embayment (RE) AOC and the Buffalo River (BR) AOC (Haynes and Wellman 2015 a, b) and E & E (2019) studies of mink prey in the EMC AOC and SA and OOC REF area were combined and analyzed to compare COC concentrations in the five areas studied by either or both Brockport and E & E (Table 6). See Electronic Appendix B, worksheet "E & E-Brkprt Mink Prey Results" for the statistical data and calculations that provided the results reported in this section.

Total Mercury

AM were collected only by Brockport in the GR portion of the RE AOC (74 [11] ng/g) and EMC SA (130 [105] ng/g), and there was no significant difference in THg concentration (ng/g) between the two areas (WRS: p = 1.0). Crayfish (ng/g) were found in the EMC AOC (35.8 [38.2]), EMC SA (30.2 [26.0]), OOC REF (13.5 [2.7]) and GR portion of the RE AOC (114.7 [16.9]), but not in the BR AOC. There were significant differences in concentrations among the four areas (KW: 0.0398; DAPC: GR AOC > EMC AOC = EMC SA > OOC REF). Concentrations of THg (ng/g) in LF differed significantly among the five areas (EMC AOC, 25.8 [20.1]; EMC SA, 26.5 [22.4]; OOC REF, 20.5 [2.1]; GR AOC, 272 [26]; BR AOC, 84.4 [8.9]) (KW: p = 0.0007; DAPC: GR AOC > BR AOC, EMC SA, EMC AOC & OOC REF). Concentrations of THg (ng/g) in UF did not differ significantly across studies (KW: p = 0.1718). Except for UF fish in the GR portion of the RE AOC (567 [44] ng/g) that slightly exceeded the 500 ng/g chronic LOAEC for THg, all TLs in the Brockport and E & E studies were below the chronic LOAEC for THg (Table 6).

Total PCB

Concentrations of TCPB in AM did not differ significantly between the GR portion of the RE AOC (4.8 [1.2] ng/g) and EMC SA (107 [28] ng/g) (WRS: p = 0.2). Concentrations (ng/g) of TPCB in the combined CR samples collected by Brockport and E & E differed significantly among the four areas sampled (EMC AOC, 489 [293]; EMC SA, 835 [975]; OOC REF, 7.9 [3.8]; GR AOC, 23.9 [4.5]) (KW: p < 0.0001; DAPC: EMC SA = EMC AOC > GR AOC = OOC REF). AM and CR did not exceed the 960 ng/g chronic LOAEC for TPCB. Concentrations (ng/g) of TPCB in LF (EMC AOC, 1,752 [623]; EMC SA. 2,902 [1084]; OOC REF, 714 [78]; GR AOC, 88 [16]; BR AOC, 381 [248]) differed significantly among areas (KW: p < 0.0001; DAPC: EMC SA = EMC AOC > BR AOC, GR AOC & OOC REF). LF exceeded the 960 ng/g chronic LOAEC for TPCB in the EMC AOC and SA. Concentrations (ng/g) of TPCB in UF (EMC AOC, 6,219 [3,382]; EMC SA, 6,911 [4,184]; OOC REF, 502 [157]; GR AOC, 332 [33]; BR AOC, 993 [184]) also differed significantly among areas for UF (KW: p = 0.0001; DAPC: EMC SA = EMC AOC > BR AOC, OOCREF & GR AOC). UF in the BR AOC slightly exceeded the chronic TPCB LOAEC and UF in the EMC AOC and SA exceeded the acute LOAEC (5,000 ng/g) for TPCB (Table 6).

PCB TEQ

Concentrations of PCB TEQ in AM did not differ significantly between the GR portion of the RE AOC (0.2 [0.3] pg/g) and EMC SA (7.2 [7.1] pg/g) (WRS: p = 0.2). Concentrations of PCB TEQ (pg/g) in the CR samples collected by Brockport and E & E (EMC AOC, 5.2 [6.7]; EMC SA, 10.4 [8.6]; OOC REF; 0.3 [0.02]; GR AOC, 0.2 [0.2]) differed significantly among the four areas (KW: p = 0.0038; DAPC: EMC SA = EMC AOC > GR AOC = OOC REF). Crayfish in the EMC SA slightly exceeded the chronic 9.2 pg/g LOAEC for PCB TEQ. Concentrations of PCB TEQ (pg/g) in LF (EMC AOC, 12.0 [10.0]; EMC SA, 44.8 [28.2]; OOC REF, 0.8 [n=1]; GR AOC, 3.3 [5.4]; BR AOC, 0.4 [0.2]) differed significantly (KW: p = 0.0002; DAPC: EMC SA = EMC AOC > BR AOC, OOC REF & GR AOC). Concentrations of PCB TEQ (pg/g) in UF (EMC AOC, 373 [312]; EMC SA, 446 [355]; OOC REF, 8.6 [0.5]; 0.8 [0.3]; BR AOC, 6.1 [3.3]) also differed significantly among areas (KW: p = 0.0002; DAPC: EMC SA = EMC AOC > OOC REF, BR AOC & GR AOC). LF and UF exceeded the chronic 9.2 pg/g LOAEC for PCB TEQ in the EMC SA and AOC. None of the samples approached the 1,000 pg/g acute LOAEC for PCB TEQ (Table 6).

BUI contaminant concentrations in water

Brockport collected whole water samples in spring, summer and fall in the EMC AOC and SA and once each season at three different locations in Lake Ontario. Concentrations (pg/mL) of THg (EMC AOC, 1.1 [1.2]; EMC SA, 1,8 [1.1]; LO, 1.3 [0.7]) did not differ significantly (KW: p = 0.5824). Concentrations (pg/mL) of TPCB (EMC AOC, 35.4 [7.2]; EMC SA, 66.8 [6.6]; LO, 0.3 [0.2]) were significantly different (KW: p = 0.0010; DAPC: EMC SA = AOC > LO). Concentrations (fg/mL) of PCB TEQ (EMC AOC, 0.16 [0.03]; EMC SA, 0.17 [0.15]; LO, 0.07 [0.07]) did not differ significantly (KW: p = 0.1016). Concentrations (fg/mL) of CDD/CDF TEQ (EMC AOC, 0.54 [0.31]; EMC SA, 0.37 [0.25]; LO, 0.48 [0.41]) also did not differ significantly (KW: p = 0.7615) among the three water bodies (Table 7; Electronic Appendix A: worksheet “Water Results”; Electronic Appendix B: worksheet “H_2O Data”).

Whole water sample data collected by the USACE in 2020-2021 and USEPA from 2005-2010 in the EMC AOC and OOC REF were analyzed for TPCB together with Brockport whole water data from EMC AOC and LO collected in 2019-2021. Concentrations (pg/mL) of TPCB in EMC AOC water (47.4 [10.3]) were significantly higher than both OOC REF (0.3 [0.1]) and LO (0.3 [0.2]) waters (KW: p < 0.0001; DAPC: EMC AOC > LO & OOC REF) (Table 7; Electronic Appendix B: worksheet “Water Results”).

Diet models

The COC concentrations found in, as well as the TLs of, prey group samples were used in diet models, which combined concentrations and TL in a proportional manner to mimic literature reports of mink diets. The diet models thus estimated mink dietary exposures to COCs found in their prey in EMC and OOC.

In the EMC SA, the typical diet including amphibians was 65% aquatic with a TL of 3.6, while the worst-case diet including amphibians was 92% aquatic with a TL of 4.6. When the

amphibians were removed from the SA diets, the typical diet was 57% aquatic with a TL of 3.6, while the worst-case diet remained at 92% aquatic with a TL of 4.7 (Table 8). As there were no amphibians in the EMC AOC, the typical diet was 57% aquatic and had a TL of 3.6, while the worst-case diet at 92% aquatic had a TL of 4.8 (Table 8).

Modeling results suggested no differences in potential dietary exposures of mink between the EMC AOC and SA (Table 8). No EMC diet models exceeded the chronic dietary LOAEC for THg (500 pg/g, Dansereau *et al.* 1999). EMC diet models for TPCB (57% and 92% aquatic) in the AOC and SA exceeded the chronic dietary LOAEC (960 ng/g, Bursian *et al.* 2006) but not the acute LOAEC (5,000 ng/g; Aulerich and Ringer 1977). All EMC diet models for PCB TEQ exceeded the chronic dietary LOAEC (9.2 pg/g, Bursian *et al.* 2006), but not the acute LOAEC (1,000 pg/g; Hochstein et al. 1998).

Diet modeling results indicated that the OOC REF had much lower potential dietary exposures for mink than the EMC AOC and SA (Table 9). None of the OOC results exceeded the chronic dietary LOAEC for THg (500 ng/g). Diet model predictions for the OOC REF were far below chronic LOAECs for TPCB and PCB TEQ. No data are known for CDD/CDF in the OOC REF. We could not calculate the TLs for OOC REF diets because we did not have TL data for the E & E (2019) fish used in this model.

Bioaccumulation models

The bioaccumulation model estimates the dietary exposure of mink to chemicals of concern based on those chemicals' concentrations in water. With the slope value a = 0.79 as recommended by Van Gestel *et al.* (1985), the minimum RSS (6.53) of the differences between the models was found at intercept b = -1.11, yielding the equation log BCF = 0.79 log Kow – 1.11 (Electronic Appendix A: Data analysis worksheet "BA Macro"). Using this equation and FCMs from Sample *et al.* (1996) and USEPA (2012a), the bioaccumulation model optimized for EMC matched the predictions of the diet model within less than 5% for typical (TL = 3.6) and TL 4 diets in EMC (Table 10). There was very close agreement of PCB congener (Figure 5) and PCB TEQ (Figure 6) concentrations predicted by the diet model and the bioaccumulation models.

Using the parameters of the bioaccumulation model as optimized for EMC, the bioaccumulation model for the OOC REF matched the OOC diet model for typical (TL = 3.6) and TL 4 diets with a mean difference of 3.2 (0.6) pg/g TEQ, just over one-third of the 9.2 pg/g chronic LOAEC, although the percent difference was much higher (94.0 [1.0] %) due to the much lower PCB TEQ concentrations in the OOC REF (Table 10).

Discussion

The overarching conclusion from the field data presented above is that, regardless of the statistical results somewhat blurred by small sample sizes, the potential biological harm to mink from concentrations of TPCB and PCB TEQ in mink prey and water in the EMC AOC and SA are an order of magnitude or greater than they are in the OOC REF, GR portion of the RE AOC

and BR AOC (Tables 5, 6 and 7). Concentrations of THg and CDD/CDF TEQ are not of biological concern to mink living in any of the five locations compared in this study.

Potential sources of error

Diet model

The diet model combines the concentrations of chemicals found in prey group samples, by incorporating them in proportions matching those of mink diets reported in the literature, to estimate the dietary exposure of mink living in areas where those prey samples were taken. The only inputs to the diet model are the COC concentrations in prey groups and the fractions of mink diet they comprise. Hence, one source of error is the uncertainty in measuring COC concentrations in prey samples, but these errors are reduced by the fractions by which they are multiplied in the diet model. Another source of error is the variation between the diet model description of mink diet based on literature values (USEPA 1993) and field conditions for mink. We have no way to quantify this potential error, but we have bounded the problem by exploring typical- and worst-case diets, with and without amphibians.

A final source of error in the OOC REF diet model (Table 9) came from using a 2.8 multiplier to convert E & E (2019) skin-on fillet concentrations for largemouth bass (Skinner et al. 2009) and northern pike (no factor provided by Skinner et al. 2009) to whole body concentrations for the same fish. Then we created composited samples of E & E fish (northern pike and largemouth bass) to match Brockport's composited samples collected in the EMC AOC and SA. Because E & E UF were collected only in the fall of 2018, while Brockport UF were collected once each in fall, spring and summer from 2018-2019, there is no way to know whether the fish sampled by the two groups were comparable in size, lipid content and, thus COC concentrations. The effect of these differences between Brockport and E & E fish samples can be seen by comparing EMC diet models in Tables 8 and 9, which show the results of using Brockport and E & E (2019) fish, respectively. Nevertheless, UF TPCB concentrations in the OOC REF (E & E fish only) were more than 10X lower than in the EMC AOC and SA (Table 6), as expected. However, this was not the case for PCB TEQ: While more than 10X lower than the concentrations in UF in the EMC AOC and SA, PCB TEQ was almost 3X higher in UF in the OOC REF than the chronic LOAEC (9.2 pg/g). We attribute the different results between TPCB (n = 6 composited samples) and PCB TEQ (n = 2 composited samples) to luck of the draw in larger vs. smaller sample sizes (Table 6).

Bioaccumulation model

Potential sources of error in the bioaccumulation model include uncertainties in values for K_{ow} and FCM, interpolations required to determine non-integer FCMs and BCFs, and non-linearity of the relationship between log K_{ow} and log BCF.

We used log K_{ow}s that were derived by computing averages across up to six values per PCB congener based on data from six different studies or models (Eisler and Belisle 1996, Hawker and Connell 1998, Jäntschi and Bolboacă 2006, Paasivirta and Sinkkonen 2009, and two

models from USEPA 2012b). The values for log K_{ow} of coplanar PCBs in those studies varied substantially; PCB 81 had the smallest log K_{ow} range (0.565) while PCB 189 log K_{ow} had the largest range (0.911). Because log K_{ow} is in the exponent of the equation $BCF = 10^{(0.79*logK_{ow}-1.06)}$, small changes in log K_{ow} create large changes in BCF and hence in BAF ($= BCF * FCM$).

We found two different sets of food chain multipliers provided by EPA (2003, 2012a, 2016), along with the explanation that FCMs vary between ecosystems (EPA 2003, Arnot and Gobas 2006). Sources of variation include characteristics of individual organisms (lipid content, diet, size, age, gender, reproductive status), species (trophic level, dietary preference, metabolic abilities) and ecosystems (temperature, water column depth, interaction of benthic species with sediment) (Arnot and Gobas 2006). We chose the available set of FCMs more likely to describe the EMC and OOC ecosystems, but without a separate study to determine the FCMs in EMC and the OOC REF, that description cannot be exact.

USEPA (2012a, 2016) recommend linear interpolation of FCMs within trophic levels, which we also had to do between trophic levels to compare with our diet model trophic levels. Linear interpolation between log K_{ow}s slightly underestimates the FCMs, as those curves are convex upward in the range of log K_{ow}s for coplanar PCBs. Differences between linear interpolations and convex cures were assumed to be negligible based on EPA's (2012a, 2016) recommendation. Linear interpolation between trophic levels also slightly underestimates the BAFs, as a best-fit curve to the FCMs of the three trophic levels for any one K_{ow} is also convex upward in the same range. The magnitude of the underestimation in the interpolation between trophic levels is shown in Figure 4 for the two PCBs with the highest toxicities, PCB 126 (log K_{ow} = 6.8) and PCB 169 (log K_{ow} = 7.4) and is also assumed to be negligible.

Arnot and Gobas (2006) did regressions to find slope, a, and intercept, b, from 392 published studies and database sources. They found that the values varied between trophic levels, i.e., autotrophs, invertebrates, and fish (trophic levels unspecified) and each had their own equations. Sources of variation are much the same as for FCMs above (Arnot and Gobas 2006), so different ecosystems will also have different equations. This finding is consistent with Van Gestel et al.'s (1985) review of ten studies all with different slopes (a) and intercepts (b), although those studies appear to be lab experiments rather than ecosystem studies.

Arnot and Gobas (2003, 2004, 2006) present a much more complex bioaccumulation model than the one used in this study that accounts for many of the sources of variation mentioned above along with others such as gill uptake and elimination, dietary uptake, fecal elimination, growth dilution, and metabolism of chemicals. They described the relationship between log BAF and log K_{ow} as "parabolic;" in their figures it is convex up with a peak at about log K_{ow} = 7.5 (Arnot & Gobas 2003). Within the range of log K_{ow} for coplanar PCBs, where 6 < log K_{ow} < 8, the slope of the curve decreases to zero, then becomes negative because the chemicals are becoming more strongly bound to DOC and POC and thus less bioavailable to the food web

(Arnot and Gobas 2003). While their model is probably more accurate, it applies to only one trophic level and requires input data that we did not have.

Our diet and bioaccumulation models show that absolute PCB TEQ concentrations are smaller in the OOC REF than in the EMC AOC and SA models by nearly two orders of magnitude (Table 10). Although we cannot quantify the errors that might occur in our bioaccumulation model, it matches the results of the diet model very well in the EMC SA and AOC. While the differences between the two models have very similar absolute size, the percent differences in OOC REF are proportionally much larger (94%) than in the EMC (4.3%); this occurs because the diet model results are much smaller in the OOC REF than in EMC AOC or SA.

Overall, these results indicate that our choices of K_{ow}s, FCMs, and *a* and *b* seem to be appropriate for the EMC and OOC REF ecosystems. *This would allow the bioaccumulation model to be used in the future as a surrogate for sampling prey, at least until the modeling results indicate that the concentrations of COCs are approaching their LOAECs, at which point another prey study could be done.*

Answers to the 12 hypotheses tested in this study

EMC AOC and SA null hypotheses

Hypothesis 1: *COC concentrations in mink prey do not differ significantly between the EMC AOC and SA*. This null hypothesis was confirmed (Table 5).

Hypothesis 2: *COC concentrations in mink prey do not differ significantly among the EMC AOC and SA, BR AOC, GR portion of RE AOC and OOC REF*. For TPCB and PCB TEQ, the EMC AOC and SA have significantly higher concentrations than the OOC REF, GR portion of the RE AOC and BR AOC. For THg, the GR portion of the RE AOC has a significantly higher concentration than the other four study sites (Table 6).

Hypothesis 3: *COC concentrations in mink prey in the EMC AOC and SA, BR AOC, GR portion of RE AOC and OOC REF are not higher than published dietary LOAECs*. THg concentrations in mink prey were below the chronic 500 ng/g chronic LOAEC in all areas except for a small exceedance by UF in the GR portion of the RE AOC. TPCB and PCB TEQ concentrations in crayfish and amphibians were below their chronic LOAECs of 960 ng/g and 9.2 pg/g, respectively, except for PCB TEQ in CR in the EMC SA that slightly exceeded the chronic LOAEC. In LF, TPCB and PCB TEQ concentrations were considerably higher than their chronic LOAECs in the EMC AOC and SA (960 ng/g and 9.2 pg/g, respectively) and well below their chronic LOAECs in the OOC REF, BR AOC and GR portion of the RE AOC. In UF, TPCB exceeded its acute LOAEC (5,000 mg/g for Arochlor 1254) in the EMC AOC and SA while TPCB in the BR AOC slightly exceeded the chronic 960 ng/g LOAEC. TPCB concentrations in the GR portion of the RE AOC and OOC REF were far lower than the chronic LOAEC. PCB TEQ exceeded the chronic LOAEC considerably in the EMC AOC and SA but not in the BR AOC and GR portion of the RE AOC. A three-fold exceedance of the chronic PCB TEQ LOAEC in the OOC REF is best

explained by small sample size (see footnote in Table 6). CDD/CDF TEQ was below its chronic LOAEC of 9.2 pg/g at all locations and contributed only 5.5 ± 4.3% to total TEQ. TPCB and PCB TEQ are by far the major COCs posing risk to mink in EMC (Table 6).

Hypothesis 4: *COC concentrations in water from EMC AOC and SA, OOC REF and LO away from tributary influences are not significantly different*. For Brockport data alone, concentrations of TPCB in the EMC AOC and SA were greater than in LO, while concentrations of THg, PCB TEQ and CDD/CDF TEQ did not differ among the three water bodies (Table 7). Using Brockport, USACE and USEPA data, TPCB concentrations in the EMC AOC were greater than in the OOC REF (Table 7).

Diet Model null hypotheses

Hypothesis 5: *COC concentrations estimated by diet models using data from the EMC AOC and SA are not significantly different*. The diet model suggested no differences in predicted dietary exposures between the EMC AOC and SA (Table 8).

Hypothesis 6: *COC concentrations estimated by diet models using data from the EMC AOC and SA are not higher than published dietary LOAECs*. No diet model predictions for EMC SA and AOC exceeded the chronic LOAEC for THg (500 ng/g). For TPCB, all diet model predictions exceeded the chronic dietary LOAEC (960 ng/g.), but none exceeded the acute dietary LOAEC (5,000 ng/g for Arochlor 1254). For PCB TEQ, all diet models exceeded the chronic dietary LOAEC (9.2 pg/g) by at least a factor of five. None of the diet models exceeded the acute dietary LOAEC for TTEQ (1,000 pg/g).

Oak Orchard Creek null hypotheses

Hypothesis 7A: *COC dietary exposure estimates for the OOC REF are not significantly different from dietary exposure estimates for the EMC SA and AOC*. The OOC REF had much lower modeled predicted dietary exposures than the EMC AOC and SA for THg, TPCB and PCB TEQ (Table 9).

Hypothesis 7B: *COC concentrations estimated by diet models using data from the OOC REF are not higher than published dietary LOAECs*. Dietary exposures of COCs in OOC REF were well below chronic LOAECs for THg, TPCB and PCB TEQ.

Bioaccumulation model null hypotheses

Hypotheses 8-9: *Predictions of the bioaccumulation models for the EMC and OOC REF will match (±20%) predictions of the EMC and OOC REF diet models*. Five diet and bioaccumulation models of PCB TEQ with different trophic levels and proportions of aquatic prey were compared for the AOC & SA in EMC and two such models were compared for the OOC REF. The absolute differences between the two sets of models' predictions were 2.6 (1.6) and 3.3 (0.6) pg/g PCB TEQ in EMC and the OOC REF, respectively. For the EMC, the diet and bioaccumulation models' predictions (the latter was optimized to match EMC diet model results) differed by 4.3 (3.3) % of the diet model

results. Predictions of the EMC model applied to the OOC REF differed by 94 (1.0) % because while the absolute differences were of the same magnitude as for EMC, the diet model results in OOC REF were two orders of magnitude smaller than in EMC (Table 10).

BUI removal criteria hypotheses relevant to this study

Hypothesis 10: *PCB concentrations in fish tissue and other prey are below thresholds likely to result in **acute** toxicity to fish or piscivorous wildlife (birds and **mammals**).* UF in the EMC AOC have a higher concentration of TPCB than the 5,000 ng/g acute LOAEC for Arochlor 1254 which is the most toxic Arochlor (Tables 5 and 6).

Hypothesis 11A: *PCB concentrations in fish tissue from comparable functional feeding groups are similar to reference sites*. TPCB and PCB TEQ concentrations in CR, LF and UF are significantly higher in the EMC AOC than in the OOC REF, GR portion of the RE AOC and BR AOC (Table 6).

Hypothesis 11B: *PCB concentrations in fish or other prey are below tissue concentrations known to cause deformities or reproductive impairment in piscivorous wildlife (birds and **mammals**).* The chronic LOAECs for TPCB and PCB TEQ known to cause deformities or reproductive impairment in mink are 960 ng/g and 9.2 pg/g, respectively. In the EMC AOC, concentrations of TPCB in LF and UF are 1.8 and 6.6 times greater, respectively, than the chronic LOAEC and concentrations of PCB TEQ in LF and UF are 1.2 and 55.4 times greater, respectively, than the chronic LOAEC (averages of values in Tables 5 and 6).

Can BUIs for the EMC AOC be removed?

In relation to their current removal criteria (Table 1), the combined field results from this study and E & E (2019) support removal of BUI #3 and do not support removal of BUI #5 in the EMC AOC. The concentration of TPCB in UF fish in the AOC exceeds the acute LOAEC used in this report (5,000 ng/g for Arochlor 1254; Aulerich and Ringer 1977) by ~24% (Table 6) while the concentration of TPCB in LF is ~35% of the acute LOAEC. Mink eating a diet high in LF and UF in the AOC are potentially at risk of consuming a lethal diet, a prospect also explored by the diet and bioaccumulation models. Considering all prey groups, the diet model for the AOC predicted that mink consuming a 57% typical aquatic diet would consume ~50% of the acute TPCB LOAEC concentration, while a mink consuming a 92% worst case aquatic diet would consume ~92% of the acute LOAEC (Table 9).

A comprehensive weight-of-evidence approach should be used by the EMC RAP Coordinating Committee to determine whether the BUIs addressed in this study can be removed using data presented in this report. First, the last two digits in an Arochlor number (except for 1016) indicate the mixture's percent chlorination which correlates well with its toxicity and means that different Arochlors have different LOAECs; e.g., 10,000 ng/g and 20,000 ng/g for Arochlors 1242 and 1016, respectively (Bleavins *et al*. 1980). Second, the typical Arochlor measured in AOC sediments has been 1248 (personal communication, Scott Pickard, USACE, Buffalo District). Thus, the mixture of Arochlors in AOC prey consumed by mink is likely

to be considerably less acutely toxic to them than 1254 alone. It is recommended that the following factors be considered when evaluating the removal of BUIs #3 and #5:

1. BUI #3—Based on the current wording of Criterion 3 of this BUI, *"PCB concentrations in fish tissue and other prey are below thresholds likely to result in **acute** toxicity to fish or piscivorous wildlife (birds and **mammals**)"*, it is unclear whether "PCB" refers to total, TEQ or both. TPCB exceeded the acute 5,000 ng/g LOAEC for Arochlor 1254 in AOC UF samples (Table 6), but not in the aquatic diet models (Tables 8 and 9). Given that other less toxic Arochlors, particularly 1248, predominate in the AOC, it is highly unlikely that a mink living in the AOC would suffer acute effects from TPCB. In support of this point, for several reasons presented earlier in this report, PCB TEQ is a much better measure of toxicity to wildlife than TPCB (Giesy and Kannan 2002). For PCB TEQ, concentrations in our mink prey samples and diet model predictions in the AOC were always below the acute LOAEC of 1,000 pg/g for PCB TEQ.

   In addition, and as discussed previously, AOC UF fish was the only prey group to exceed the acute LOAEC for TPCB (5,000 ng/g, Arochlor 1254). Projections including data from all prey groups employed in our models predict that the TPCB would fall below this LOAEC.

   Based on this information, a mink consuming aquatic prey in the EMC AOC would be highly unlikely to suffer acute toxicity from TPCB or PCB TEQ, and Criterion 3 for BUI #3 is recommended for removal in the EMC AOC.

2. BUI #5—Criterion 1 of this BUI, *"PCB concentrations in fish tissue from comparable functional feeding groups are similar to reference site(s)"*, is not recommended for removal based on the results of this study. PCB (total and TEQ) concentrations in the AOC are significantly higher than in two other AOCs (BR AOC; GR portion of the RE AOC) and the OOC REF.

3. BUI #5—Criterion 2 of this BUI, *"PCB concentrations in fish and other prey are below tissue concentrations known to cause deformities or reproductive impairment* [chronic effects] *in piscivorous wildlife."* The currently established chronic TPCB and PCB TEQ LOAECs for deformities and reproductive impairment in piscivorous wildlife, of which mink are the most sensitive species in North America, are 960 ng/g and 9.2 pg/g TEQ, respectively (Bursian *et al.* 2006). In the AOC, TPCB concentrations in LF and UF exceeded the chronic LOAEC by factors of 1.2 and 6.6, respectively. Similarly, PCB TEQ exceeded its chronic LOAEC by factors of 1.2 and 55.4, respectively. Diet model predictions for the AOC and SA (averages of the five values in Tables 8 and 9) exceeded the chronic TPCB and PCB TEQ LOAECs by factors of 3.4 and 6.5, respectively. Accordingly, a mink living in the AOC would be likely to suffer deformities and reproductive impairment and this BUI is not recommended for removal.

Other than waiting decades or centuries for natural ecosystem processes to bury or degrade PCBs in upstream source areas, the lowering of water PCB concentrations and, thus, fish

tissue PCB concentrations to levels in the AOC that would allow removal of either BUI #5 Criterion 1 OR Criterion 2 would require accurate identification and remediation of those source areas, an expensive prospect. Upstream areas are currently being evaluated by the USEPA through the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA/ Superfund) process. Remedial design for the Creek Corridor (stream reach OU2) and remedial investigation from the city of Lockport to Lake Ontario (stream reach OU3) are underway, including a Human Health Risk Assessment (HHRA) and Baseline Ecological Risk Assessment (BERA), and proposed remedial alternatives for the creek and its floodplain are nearing completion (Eighteen Mile Creek Superfund Site, www.epa.gov/superfund/eighteenmile-creek and click "Site Documents & Data").

Recommendations and Conclusions

1. Based on the results this study, BUI #3, Criterion 3, *"PCB concentrations in fish tissue [UF in the EMC AOC] are below thresholds likely to result in **acute** toxicity to fish or piscivorous wildlife (birds and **mammals**)"* is true and is recommended for removal in the EMC AOC. Although the concentration of TPCB in the UF prey group in the AOC exceeds the acute LOAEC when considered in isolation, weight-of-evidence indicates that PCBs in the AOC are not likely to cause acutely toxicity in mink.
2. Based on the results of this study, BUI #5, Criteria 1 "*PCB concentrations in fish tissue from comparable functional feeding groups are similar to reference sites*" OR 2 "*PCB concentrations in fish or other prey are below tissue concentrations known to cause deformities or reproductive impairment in piscivorous wildlife*" are not recommended for removal. Concentrations of TPCB and PCB TEQ are significantly higher in the EMC AOC than the GR portion of the RE AOC, BR AOC and OOC REF (Criterion 1), and concentrations of TPCB and PCB TEQ in the AOC substantially exceed their chronic LOAECs (Criterion 2).
3. A better alternative to looking at tissue concentrations in separate trophic groups to "guestimate" whether mink may be adversely affected chronically (health) or acutely (lethal) in the AOC would be to consider the results of the diet and bioaccumulation models used in this study that reflect literature-based typical- and worst-case aquatic diets and bioconcentration of PCB TEQ from water based on co-planar PCB k_{ow}s for mink living in riverine-lacustrine habitats like the AOC. By considering all trophic levels in the mink diet, concentrations of TPCB predicted for their tissue are near (92% aquatic diet) or about half (57% aquatic diet) of the most conservative (in terms of protecting mink health) 5,000 ng/g acute LOAEC for Arochlor 1254 (Aulerich and Ringer 1977). For PCB TEQ, modeled concentrations ranged by factors of 4.0 to 9.2 higher than the 9.2 pg/g chronic LOAEC. Given that exposure to TEQ PCB by aquatic biota is a better way to determine risk to mink than exposure to TPCB (Giesy and Kannon 2002), perhaps the

independently modeled PCB TEQ data in Tables 8 and 9 are what the RAP Coordinating Committee should weigh most highly while considering whether to remove BUI #5.

Relevant to BUI #5 Removal Criterion 2, getting PCB TEQ, not TPCB, below chronic LOAECs in contaminated ecosystems is now considered the best way to protect the health of piscivorous birds and mammals. In the future, the RAP Coordinating Committee should consider using water sampling and our bioaccumulation model that was optimized for the EMC AOC to predict PCB TEQ in mink. Given currently high TEQ in mink prey it will take many years for predicted PCB TEQ to fall below the LOAEC, at which time another mink prey study should be conducted so that then existing PCB TEQ concentrations in mink prey can be used in our diet model. If the bioaccumulation and diet models at that time agree that PCB TEQ concentrations are less than the chronic LOAEC, BUI #5 Removal Criterion 2 would be satisfied.

An alternative to the approach described above would be to locate and remediate source areas in EMC to reduce PCB concentrations in water and, subsequently, mink prey in the AOC below the chronic LOAECs for mink, which would be a long and costly process.

4. Another approach for the RAP Coordinating Committee to consider would be to examine the findings reported in the mink habitat suitability and signs portion of this study (Haynes and Wellman 2019) that led the project team to decide that a mink prey study was the only way to address BUIs #3 and #5. Mink habitat suitability was low, only one definitive mink sign (tracks in mud, ~100 m below Burt Dam) was observed, and the area of the AOC is so small that only 1-2 male mink at a time could hold territories there. While some mink may pass through the AOC to reach other habitats, the AOC itself cannot sustain a viable mink population and the same is true for this study's "source area" between Ide Road and Burt Dam (Figure 1). While any mink living long-term in the AOC would exceed the chronic LOAEC for PCB TEQ, the RAP Coordinating Committee should consider removing BUI #5, Criterion 2 on the basis that few or no mink can be long-term residents of the AOC due to habitat quality and area constraints.

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Tables

Table 1. EMC AOC removal criteria for BUIs 3 and 5 as of 03/21/21.

BUI 3. Degradation of Fish and Wildlife Populations

Status: Impaired

Removal Criteria: Fish community metrics (e.g., diversity, abundance, biomass, and condition) are similar to reference site(s); **AND**

Benthic macroinvertebrate community composition is within the range expected and similar to reference site condition; **AND**

PCB concentrations in fish tissue and other prey are below thresholds likely to result in acute toxicity to fish or piscivorous wildlife (birds and mammals).

BUI 5. Bird or Animal Deformities or Reproductive Problems

Status: Impaired

Removal Criteria: PCB concentrations in fish tissue from comparable functional feeding groups are similar to reference site(s); **OR**

PCB concentrations in fish and other prey are below tissue concentrations known to cause deformities or reproductive impairment in piscivorous wildlife.

Table 2. Definitions of acronyms used in this report.

AhR: Aryl hydrocarbon Receptor
AM: Amphibian
Acute LOAEC: Lowest Observed Adverse Effect (dietary) Concentration that kills an organism
AOC: Area of Concern
BAF: Bioaccumulation Factor (diet to tissue)
BCF: Bioconcentration Factor (water to tissue)
BERA: Baseline Ecological Risk Assessment
BR AOC: Buffalo River AOC
BUI: Beneficial Use Impairment
CDD: Chlorinated Dibenzo Dioxins
CDF: Chlorinated Dibenzo Furans
CERCLA: Comprehensive Environmental Response, Compensation and Liability Act ("Superfund")
Chronic LOAEC: Lowest Observed Adverse Effect (dietary) Concentration that harms an organism
COC: Chemical of Concern
CR: Crayfish
DAPC: Dunn's All-Pairwise Comparison
EMC: Eighteenmile Creek (Area of Concern = AOC and Source Area = SA)
FCM: Food Chain Multiplier
GR AOC: Genesee River portion of the Rochester Embayment AOC
HHRA: Human Health Risk Assessment
IJC: International Joint Commission
KW: Kruskal-Wallis AOV (Analysis of Variance of Ranks)
LF: Lower Trophic Level Fish
LMB: Largemouth Bass
Log K_{ow}: Logarithm of the octanol-water partition coefficient of a chemical
OOC REF: Oak Orchard Creek Reference Area
NP: Northern Pike
PCB: Polychlorinated Biphenyls
RAP: Remedial Action Plan
TEF: Toxic Equivalency Factor
TEQ: Toxic Equivalents
THg: Total Mercury
TL: Trophic Level
UF: Upper Trophic Level Fish
USACE: U.S. Army Corps of Engineers
USEPA: U.S. Environmental Protection Agency
WRS: Wilcoxon Rank Sum Test

Table 3. Dates and locations of water and biological sampling.

	Spring	Summer	Fall
Water (1 gal. each)			
EMC			
Source Area	2019: 5/16	2019: 7/29	2018: 10/10
AOC	2019: 5/15	2019: 7/31	2018: 10/10
Lake Ontario	2019: 5/15 (off Eighteenmile Creek)	2019: 8/5 (off Braddock Bay)	2020: 10/13 (off Sandy Creek)
OOC (USACE)	2021: 3/16	2020: 8/10	
Mink Prey			
Amphibians[a]			
Source Area	2019: 5/23-24 (10)	2019: 7/29 (0)	2018: 9/22 (0)
	2020: 3/26-4/8 (10)		2019: 9/25 (4 juv.)
AOC	2020: 5/20 (1)	2019: 7/31 (0)	2018: 9/22 (0)
Crayfish[b]			
Source Area	2019:5/23 (~40)	2019: 7/29 (~30)	2018: 9/22 (42)
AOC	2020: 5/20 (32)	2019: 7/31 (39)	2018: 9/21 (33)
Fish[c]			
Source Area	2019: 5/16 (15)	2019: 7/29 (15)	2019: 9/25 (15)
AOC	2019: 5/15 (15)	2019: 7/31 (15)	2018: 10/6 (15)
Oak Orchard Creek			
Crayfish[b]	2020: 6/4 (40)	2020: 7/30 (50)	2020: 10/2 (40)

[a]In the Source Area, adult frogs and toads were seen only in the spring; no frogs were seen in the summer and only four, very small young-of-the-year frogs were seen in the fall. In the AOC, only two toads were seen across the three seasons sampled. Species of frogs collected were leopard frog (*Lithobates pipiens*), green frog (*L. clamitans*) and American toad (*Anaxyrus americanus*)

[b]The species of crayfish collected was the Northern clearwater crayfish, *Orconectes propinquus*.

[c]Species of lower trophic level fish collected across three seasons (N=60) were mostly bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*), plus six yellow perch (*Perca flavescens*) and one rock bass (*Ambloplites rupestris*). Species of upper trophic level fish collected across three seasons (N=30) were mostly largemouth bass (*Micropterus salmoides*) and smallmouth bass (*M. dolomieu*), plus four northern pike (*Exox lucius*).

Table 4. Fishes caught for chemical analysis in Eighteenmile Creek during the mink prey study.

		Season		
Location	**Spring**	**Summer**	**Fall**	**Percent**
Upper Trophic Level				
Area of Concern				
Northern Pike	1		1	13.3%
Largemouth Bass	2	3	4	60.0%
Smallmouth Bass	2	2		26.7%
Source Area				
Northern Pike	1			6.7%
Largemouth Bass	4	2	5	73.3%
Smallmouth Bass		3		20.0%
Lower Trophic Level				
Area of Concern				
Bluegill	4	4	3	36.7%
Pumpkinseed	6	4	4	46.7%
Yellow Perch		1	3	13.3%
Rock Bass		1		3.3%
Source Area				
Bluegill	10	5	6	70.0%
Pumpkinseed		3	3	20.0%
Yellow Perch		2	1	10.0%

Table 5. Mean (SD) trophic level and concentrations of chemicals of concern of mink prey collected in this study.

Numbers in red exceed chronic LOAECs: 500 ng/g THg; 960 ng/g TPCB; 9.2 pg/g of PCB, CDD or CDF TEQ, combined or separately. Numbers in **red bold** exceed acute LOAECs: 5,000 ng/g TPCB. NSC = No Samples Collected.

Category	Crayfish Mean (SD)	P	Result	Lower TL Fish Mean (SD)	P	Result	Upper TL Fish Mean (SD)	P	Result	Amphibians Mean (SD)
Trophic Level										
EMC AOC	3.98 (0.06)			4.82 (0.81)			5.14 (0.27)			ND
EMC SA	3.80 (0.21)		OOC>SA;	4.43 (0.11)		AOC>SA	5.20 (0.06)			2.5 (0.1)
OOC REF	4.37 (0.06)	0.0067	AOC=SA&OOC	NSC	0.854	suggested	NSC	0.7	AOC=SA	NSC
Total Mercury (ng/g)										
EMC AOC	35.8 (38.3)			50.1 (31.2)			215.3 (71.9)			NSC
EMC SA	30.2 (26.0)			64.3 (12.6)			234.3 (58.1)			129.5 (105.4)
OOC REF	13.5 (2.7)	0.6262	AOC=SA=OOC	NSC	1	AOC=SA	NSC	1	AOC=SA	NSC
Total PCB (ng/g)										
EMC AOC	293 (170)			619 (534)			**6,395** (4,977)			NSC
EMC SA	1,210 (1,413)		SA>OOC;	2,307 (950)			3,830 (468)			107.2 (77.5)
OOC REF	7.7 (3.7)	0.0067	AOC=SA&OOC	NSC	0.2	AOC=SA	NSC	0.7	AOC=SA	NSC
PCB TEQ (pg/g)										
EMC AOC	5.2 (6.7)			12.2 (15.7)			136.7 (158.7)			ND
EMC SA	10.4 (8.6)		SA>OOC;	57.0 (44.4)			164.7 (118.5)			7.2 (7.1)
OOC REF	0.3 (0.02)	0.0155	AOC=SA&OOC	NSC	0.2	AOC=SA	NSC	1	AOC=SA	NSC
CDD/CDF TEQ (pg/g)										
EMC AOC	0.7 (0.2)			0.7 (0.5)			3.4 (2.1)			NSC
EMC SA	0.6 (0.5)			1.8 (1.0)			2.3 (1.8)			0.9 (0.8)
OOC REF	0.07 (0.02)	0.7	AOC=SA=OOC	NSC	0.1	AOC=SA	NSC	0.7	AOC=SA	NSC
Total TEQ (pg/g)										
EMC AOC	5.9 (6.8)			12.9 (16.0)			140.2 (159.0)			NSC
EMC SA	11.0 (8.3)		SA>OOC	58.9 (43.5))	0.2	AOC=SA	167.0 (118.9)	1	AOC=SA	8.1 (7.0)
OOC REF	0.4 (0.06)	0.0155	AOC=SA&OOC	NSC			NSC			NSC

Table 6. Mean (SD) concentrations of chemicals of concern collected by Brockport (2013-2014) and E & E (2019).
Numbers in red exceed chronic LOAECs: 500 ng/g THg; 960 ng/g TPCB; 9.2 pg/g total PCB/CDD/CDF TEQ combined or separately. Numbers in **red bold** exceed acute LOAECs: 5,000 ng/g TPCB. NSC = No Samples Collected.

Category	Lower TL Fish Mean (SD)	P	Result	Upper TL Fish Mean (SD)	P	Result
Total Mercury (ng/g)						
EMC AOC	25.8 (20.1)			279 (130)		
EMC SA	26.5 (22.4)			310 (117)		
OOC REF	20.5(2.1)			384 (106)		
GR AOC	272 (26)		GRAOC>BRAOC=EMCSA=	567 (44)		EMCAOC=EMCSA> BRAOC=
BR AOC	84.4 (8.9)	0.0007	EMCAOC=OOCREF	265 (112)	0.1718	GRAOC= OOC REF
Total PCB (ng/g)						
EMC AOC	1,752 (623)			**6,219** (3,382)		
EMC SA	2,902 (1,084)			**6,911** (4,184)		
OOC REF	714 (78)			502 (157)		
GR AOC	88 (16)		EMCSA=EMCAOC>	332 (33)		EMCAOC=EMCSA>BRAOC=GRAOC=
BR AOC	381 (248)	<0.0001	BRAOC=GRAOC=OOCREF	993 (184)	0.0001	OOC REF
PCB TEQ (pg/g)						
EMC AOC	12.0 (10.0)			373 (312)		
EMC SA	48.8 (28.2)			446 (355)		
OOC REF	0.8 (n=1)			8.6 (0.5) (n=2)		EMCSA=EMCAOC>GRAOC
GR AOC	3.3 (5.4)		EMCSA=EMCAOC>			EMCSA=EMCAOC=OOCREF=BRAOC
BR AOC	0.4 (0.2)	0.0002	GRAOC=OOCREF=BRAOC	6.1 (3.3)	0.0002	OOCREF=BRAOC=GRAOC

Table 6 continues on next page

Category	Crayfish Mean (SD)	P	Result	Amphibians Mean (SD)	P	Result
Total Mercury (ng/g)						
EMC AOC	35.8 (38.2)			NSC		
EMC SA	30.2 (26.0)			130 (105)		
OOC REF	13.5 (2.7)			NSC		
GR AOC	114.7 (16.9)		GRAOC>EMCAOC=	74 (11)		
BR AOC	NSC	0.0398	EMCSA>OOCREF	NSC	1.0	EMCSA=GRAOC
Total PCB (ng/g)						
EMC AOC	489 (293)			NSC		
EMC SA	835 (975)			107 (28)		
OOC REF	7.9 (3.8)		EMCSA=EMCAOC >	NSC		
GR AOC	23.9 (4.5)	<0.0001	GRAOC=OOCREF	4.8 (1.2)	0.2	EMCSA=GRAOC
BR AOC	NSC			NSC		
PCB TEQ (pg/g)						
EMC AOC	5.2 (6.7)			NSC		
EMC SA	10.4 (8.6)			7.2 (7.1)		
OOC REF	0.3 (0.02)		EMCSA=EMCAOC >	NSC		
GR AOC	0.2 (0.2)	0.0038	GRAOC=OOCREF	0.2 (0.3)	0.2	EMCSA=GRAOC
BR AOC	NSC			NSC		

Table 7. Mean (SD) chemical of concern concentrations in whole water collected during this study and by USACE[a] and USEPA[b].

Brockport Data	**Mean (SD)**	**P**	**Result**
THg (pg/mL)			
EMC AOC	1.1 (1.2)		
EMC SA	1.8 (1.1)		
L. Ontario	1.3 (0.7)	0.5824	EMCAOC=EMCSA=LO
Total PCB (pg/mL)			
EMC AOC	35.4 (7.2)		
EMC SA	66.8 (6.6)		
LO	0.3 (0.2)	0.0010	AOC = SA > LO
PCB TEQ (fg/mL)			
EMC AOC	0.16 (0.03)		
EMC SA	0.17 (0.15)		
L. Ontario	0.07 (0.07)	0.1016	EMCAOC=EMCSA=LO
CDD/CDF TEQ (fg/mL)			
EMC AOC	0.54 (0.31)		
EMC SA	0.37 (0.25)		
L. Ontario	0.48 (0.41)	0.7615	EMCAOC=EMCSA=LO
USEPA, USACE & Brockport Data			
TPCB (pg/mL)			
EMC AOC	47.4 (10.3)		
OOC REF	0.29 (0.1)		
L. Ontario	0.3 (0.2)	<0.0001	EMC AOC > OOC REF = LO

[a]Data for fall 2020 and spring 2021 were provided by Andrew Lenox, USACE, Buffalo, NY District.

[b]Data for 2005-2010 were in USEPA. 2011. Final Data Report, Lake Ontario Tributaries, 2009-2010 (Report provided by Andrew Lenox, USACE, Buffalo District).

Table 8. Diet model estimates of mink exposures in EMC. Values in red for TPCB (Arochlor 1254) and PCB TEQ exceed their chronic LOAECs. No TPCB and PCB TEQ values exceed the acute LOAEC. All diets were derived from composited samples of crayfish, lower trophic level fish (bluegill and pumpkinseed) and upper trophic level fish (largemouth bass and northern pike) collected for this study.

	Trophic Level	THg (ng/g)	Total PCB (ng/g)	TEQ from PCB (pg/g)	TEQ from CDD/CDF (pg/g)
LOAECs					
Chronic		500	960	9.2	9.2
Acute		1,000	5,000	1,000	1,000
DIET					
SA with Amphibians					
65% Aquatic	3.6	100.3	1,719	64.2	1.1
TL = 4	4.0	121.4	2,226	85.6	1.4
92% Aquatic	4.6	157.5	2,888	111.0	1.9
SA No Amphibians					
57% Aquatic	3.6	89.8	1,710	63.6	1.1
TL = 4	4.0	119.1	2,237	85.9	1.4
92% Aquatic	4.7	158.8	2,982	114.5	1.9
AOC No Amphibians					
57% Aquatic	3.6	82.1	2,243	47.7	1.3
TL = 4	4.0	104.4	2,897	61.7	1.6
92% Aquatic	4.8	143.7	3,989	84.9	2.3

Table 9. Diet model comparison of EMC with OOC.
Values in red exceed the chronic LOAEC for TPCB (Arochlor 1254) and PCB TEQ. The **red bold** value exceeds the acute LOAEC for Arochlor 1254. Diets are based on composited samples of crayfish and pumpkinseed collected for this study and by E & E (2019), respectively. Largemouth bass fillet data from E & E (2019) were converted to composited whole-fish data before multiplying by a correction factor of 2.8 (Skinner *et al*. 2009).

	THg (ng/g)	**Total PCB** (ng/g)	**TEQ from PCB** (pg/g)
LOAECs			
Chronic	500	960	9.2
Acute	1000	5,000	1000
DIET			
EMC SA			
57% Aquatic	117	3,126	80.0
92% Aquatic	196	**5,563**	142.9
EMC AOC			
57% Aquatic	124	2,561	37.0
92% Aquatic	217	4,619	65.9
OOC			
57% Aquatic	140	11	0.1
92% Aquatic	250	22	0.2

Table 10. Comparison of diet model and bioaccumulation model estimates of mink dietary exposure to PCB TEQ[a] (pg/g).

		EMC SA		EMC AOC		OOC REF[a]	
Amphibians Included	Yes	No	No	No	No	No	No
Trophic Level	3.6	3.6	4.0	3.6	4.0	3.6	4.0
% Aquatic Prey	65	57	69	57	67	57	65
Diet Model PCB TEQ (pg/g)	64.2	63.6	85.9	47.7	61.7	3.0	3.8
Bioaccumulation Model PCB TEQ (pg/g)	66.6	59.6	87.9	43.5	62.0	0.2	0.2
Absolute Difference (pg/g)[b]	2.4	4.0	2.0	4.2	0.3	2.8	3.6
% Difference[c]	3.7	6.2	2.3	8.8	0.5	93.3	94.7

[a]OOC REF bioaccumulation was calculated using the model optimized for EMC SA and AOC based on samples we collected in EMC. The OOC REF diet model used data from crayfish collected in our study along with composited pumpkinseed and largemouth bass fillet samples (converted to whole fish concentrations per Skinner *et al.* 1996) collected by E & E (2019).

[b]Absolute difference between diet and bioaccumulation models: EMC mean (SD) = 2.6 (1.6) pg/g; OOC REF mean (SD) = 3.2 (0.6) pg/g

[c]Difference between models as percent of the diet model result: EMC mean (SD) = 4.3 (3.3) %; OOC REF mean (SD) = 94.0 (1.0) %.

Figures

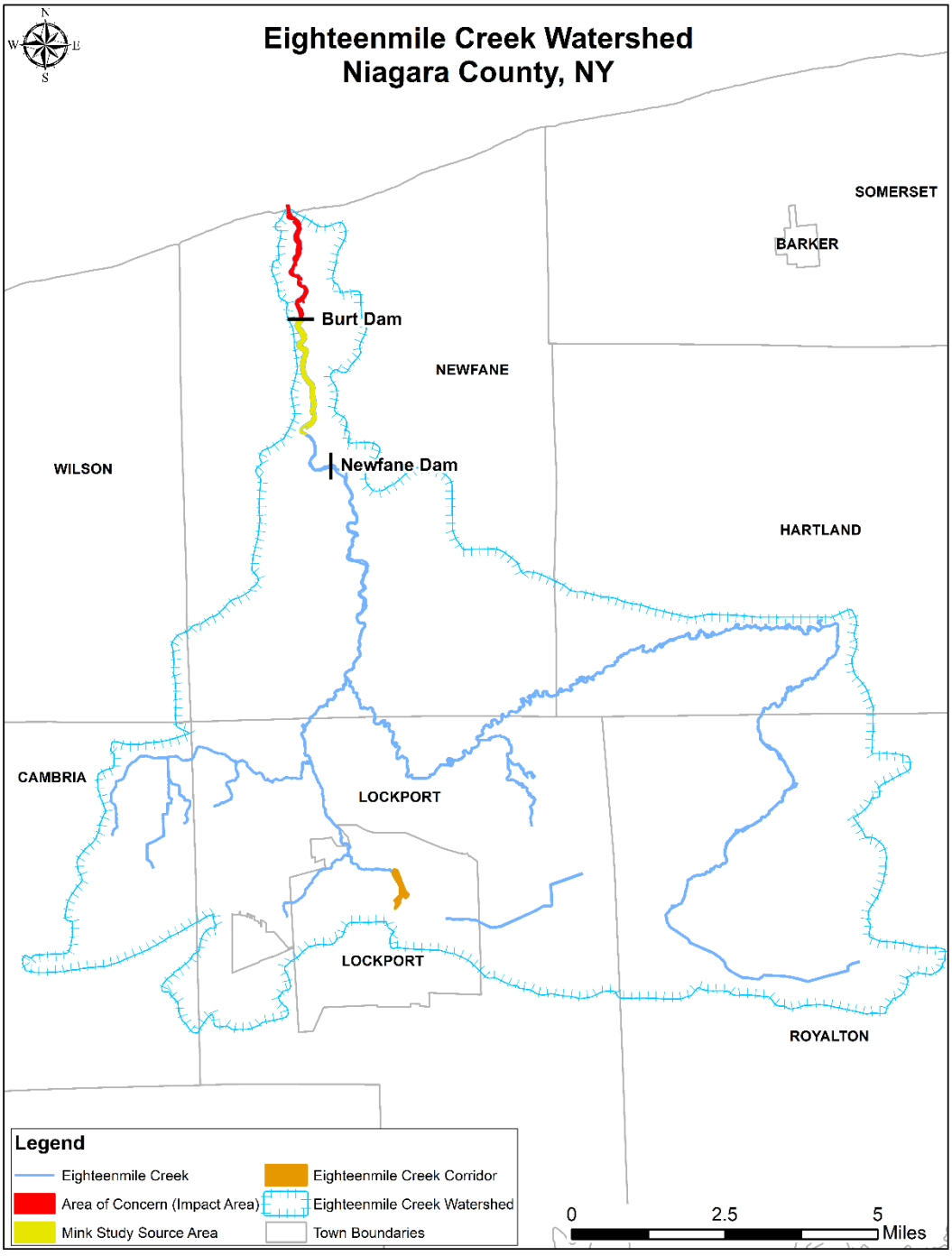


Figure 1. Map of the Eighteenmile Creek watershed.
For this project, the portion of the Source Area sampled was from Burt Dam to ~1 mi. below Newfane Dam. Map created by Scott Collins, Niagara County Soil and Water Conservation District, Lockport, NY.

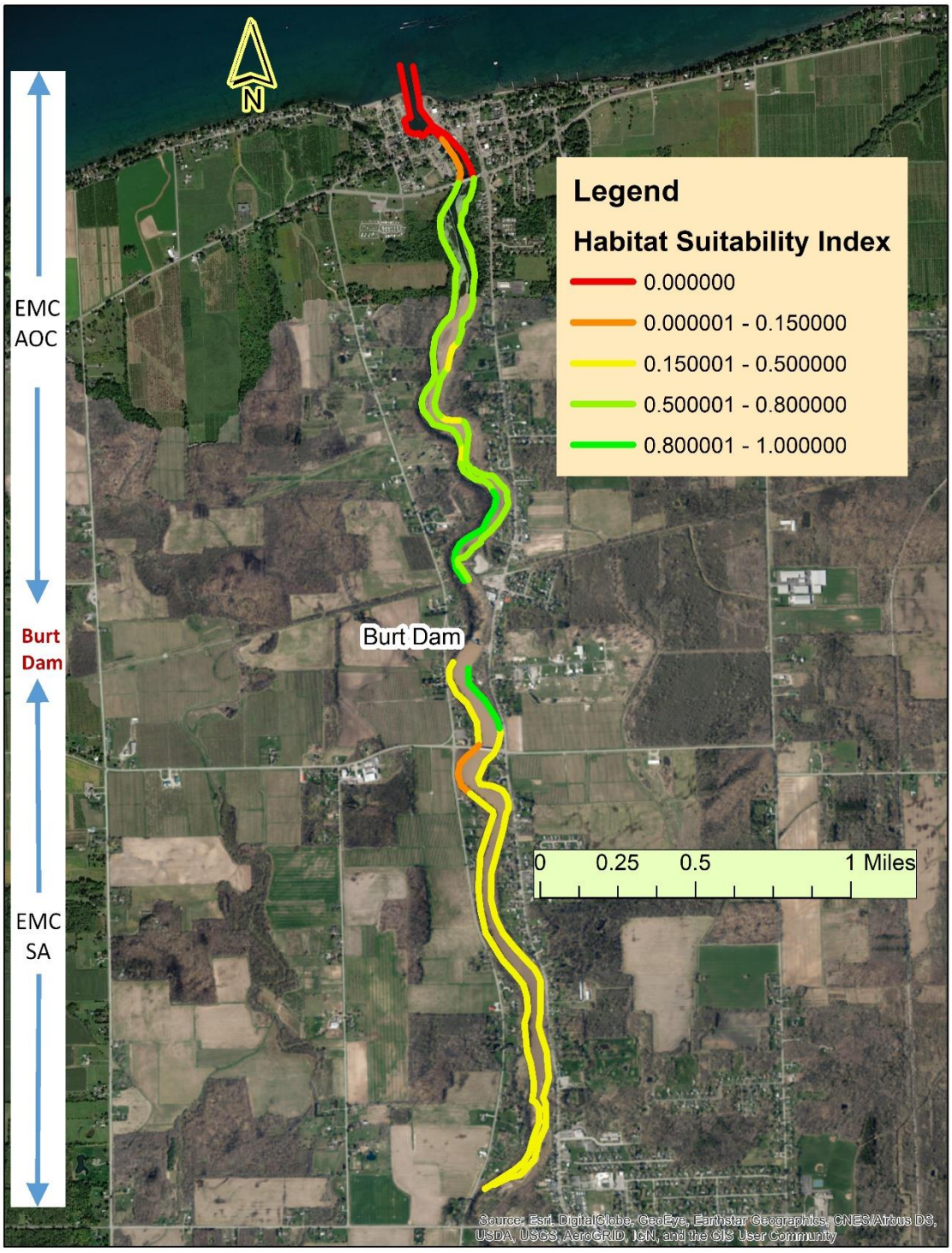


Figure 2. Mink habitat suitability index (HSI) scores.

The AOC extends north of Burt Dam to Lake Ontario and the Source Area extends south of Burt Dam to Ide Road which runs east-west along the bottom of the map. Colored lines indicate HSI scores.

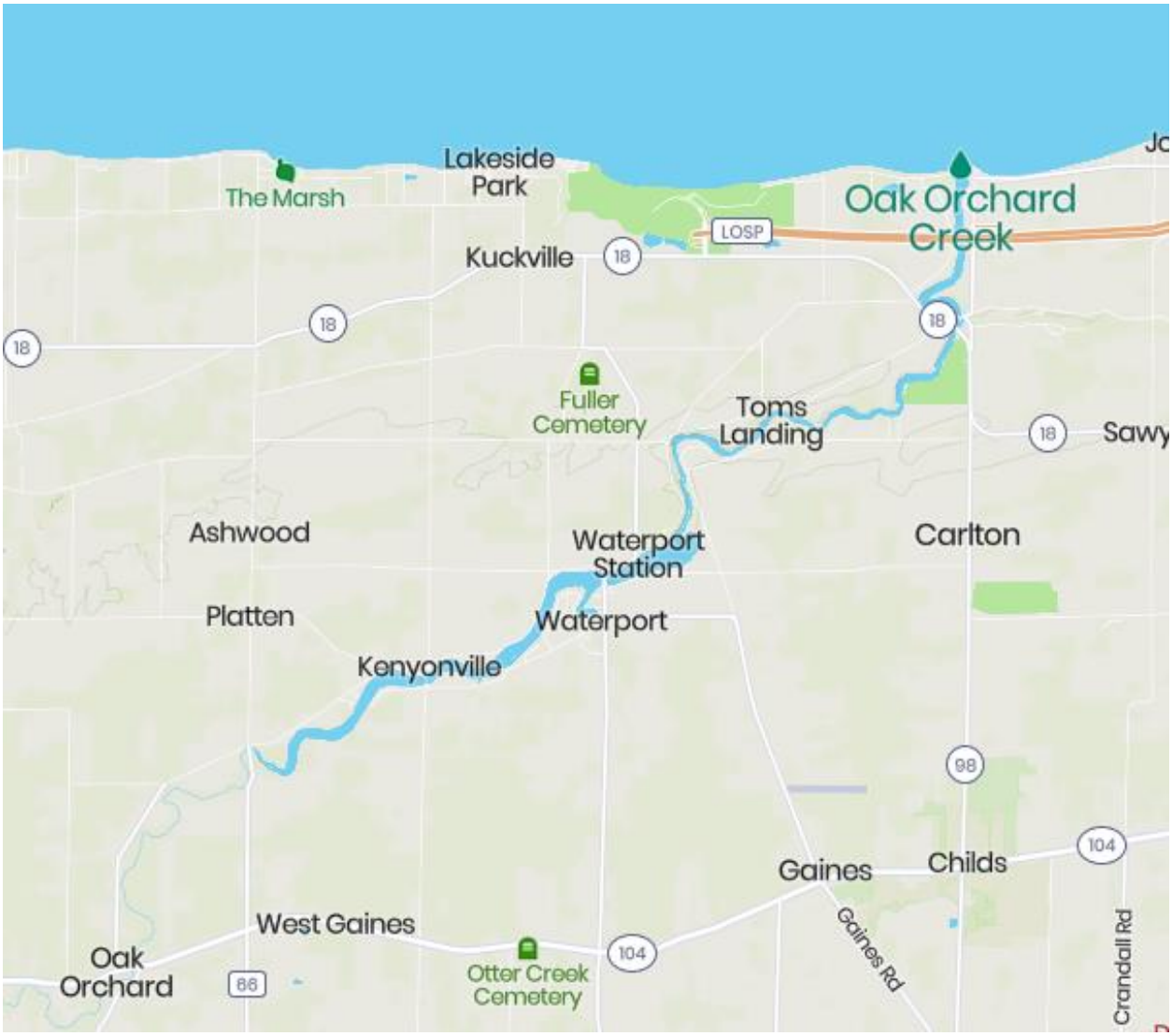


Figure 3. Map of the Oak Orchard Creek watershed.

For this project, Brockport sampled crayfish in the portion of Oak Orchard Creek that extended ~1 mi. below the Waterport Station Dam.

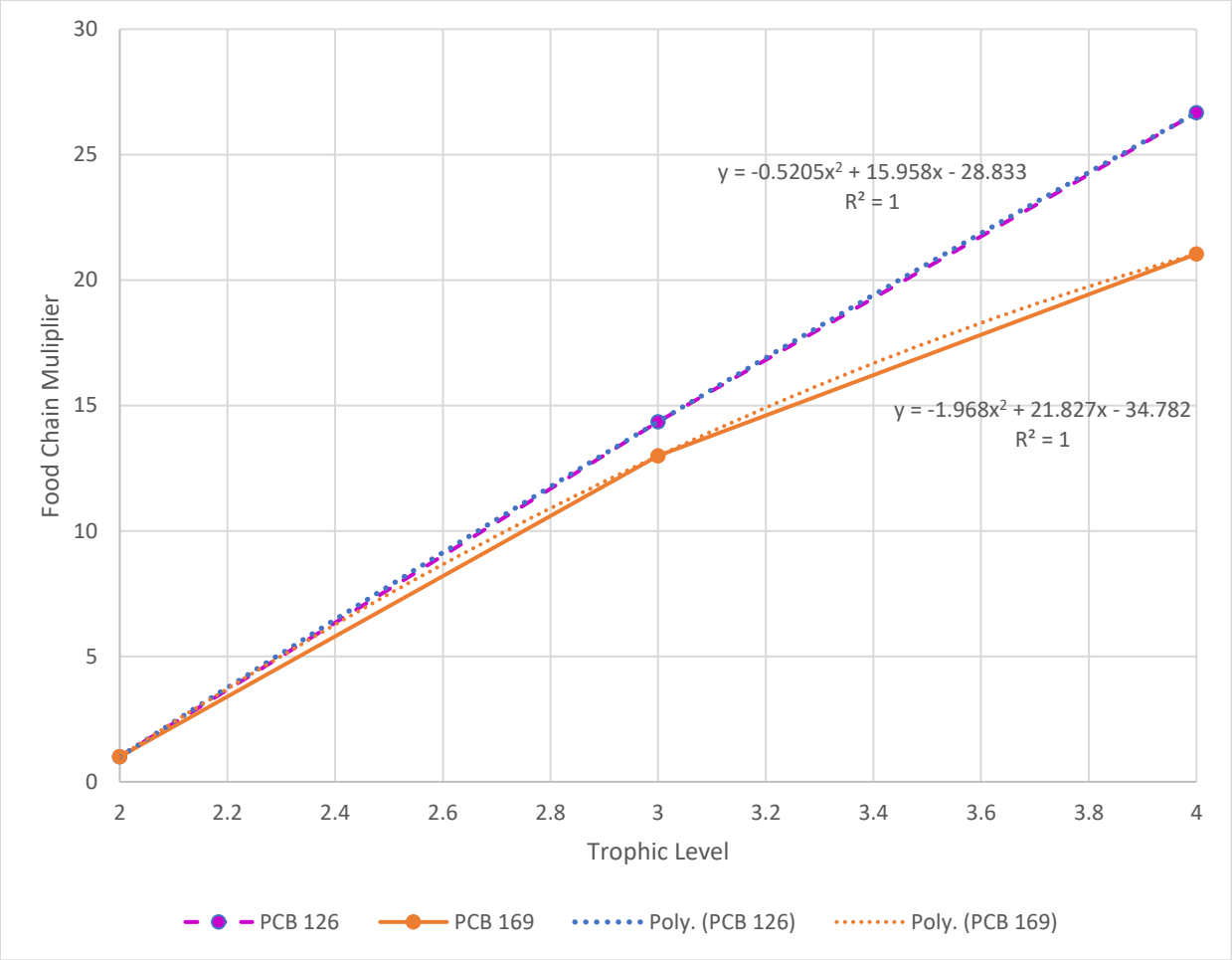


Figure 4: Non-linearity of Food Chain Multipliers vs. Trophic Level.
FCMs had to be interpolated for diets with trophic level of 3.6. This was done indirectly inside the bioaccumulation model.

Figure 5. Correlation (R = 0.73) of bioaccumulation model and diet model predictions for PCB congener concentrations.

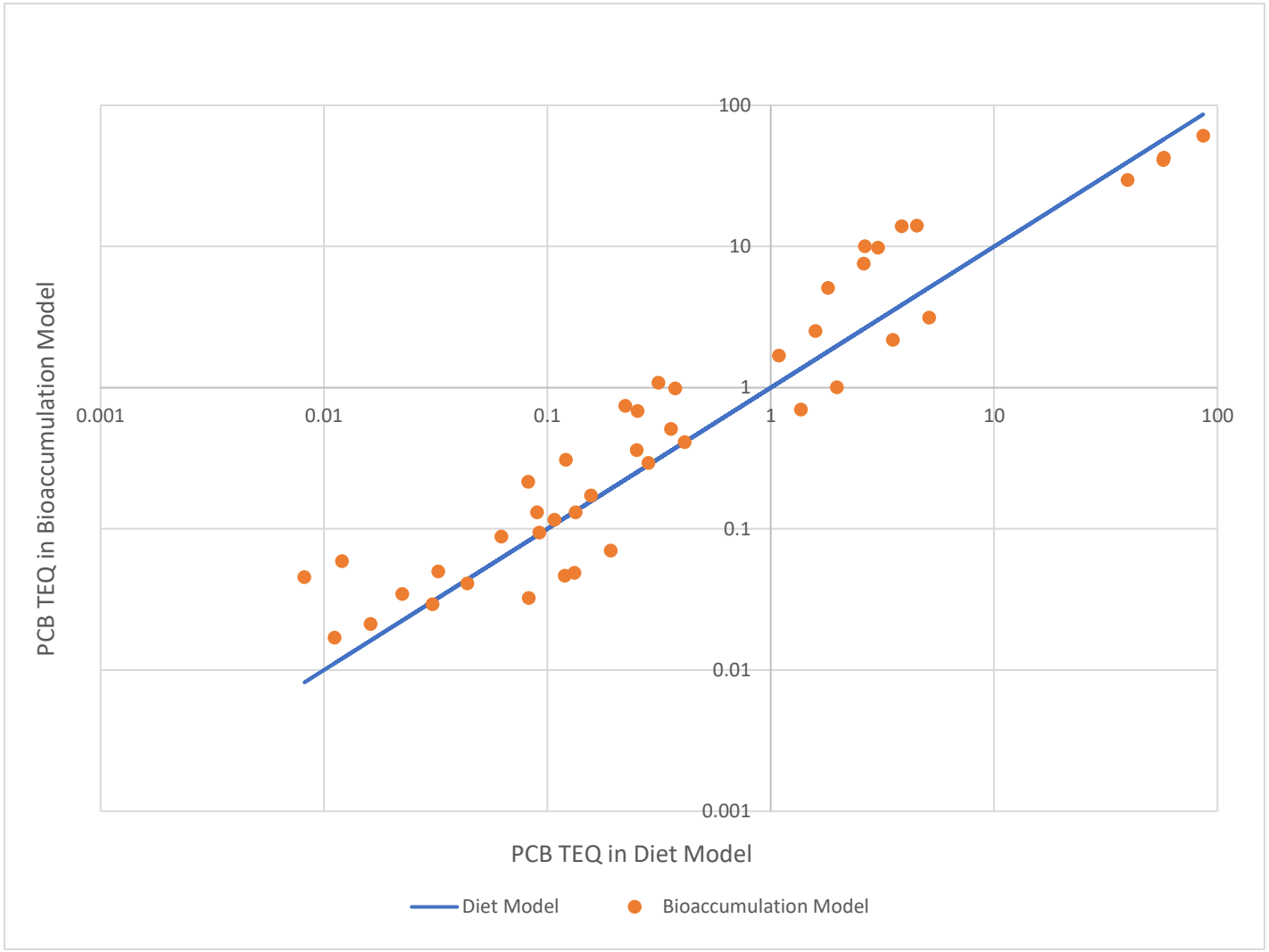


Figure 6. Correlation (R = 0.98) of bioaccumulation model and diet model predictions for PCB congener TEQ.

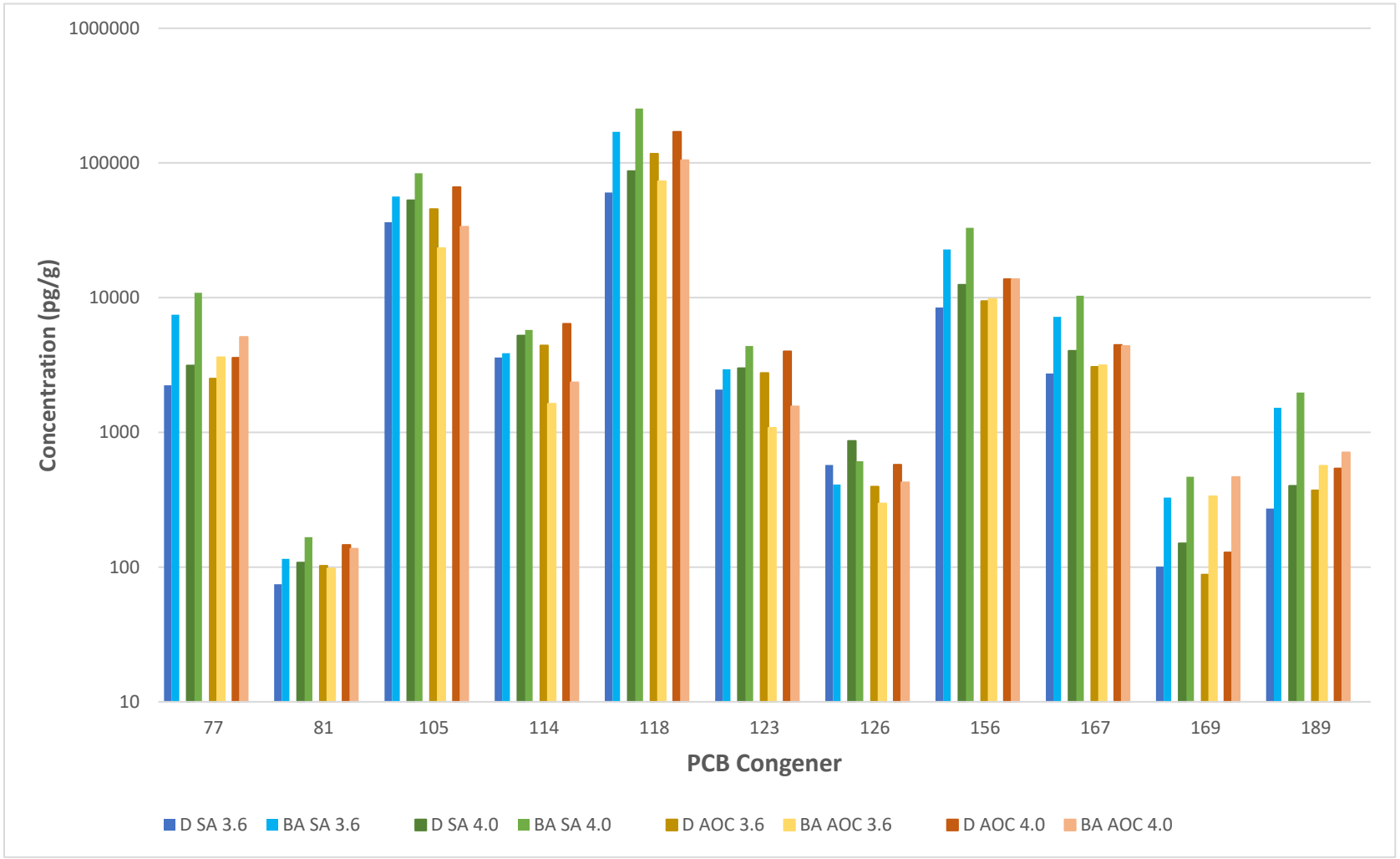
Appendices

Appendix A: Chemical Data and Modeling Calculations (electronic).

Appendix B: Statistical Calculations (electronic).

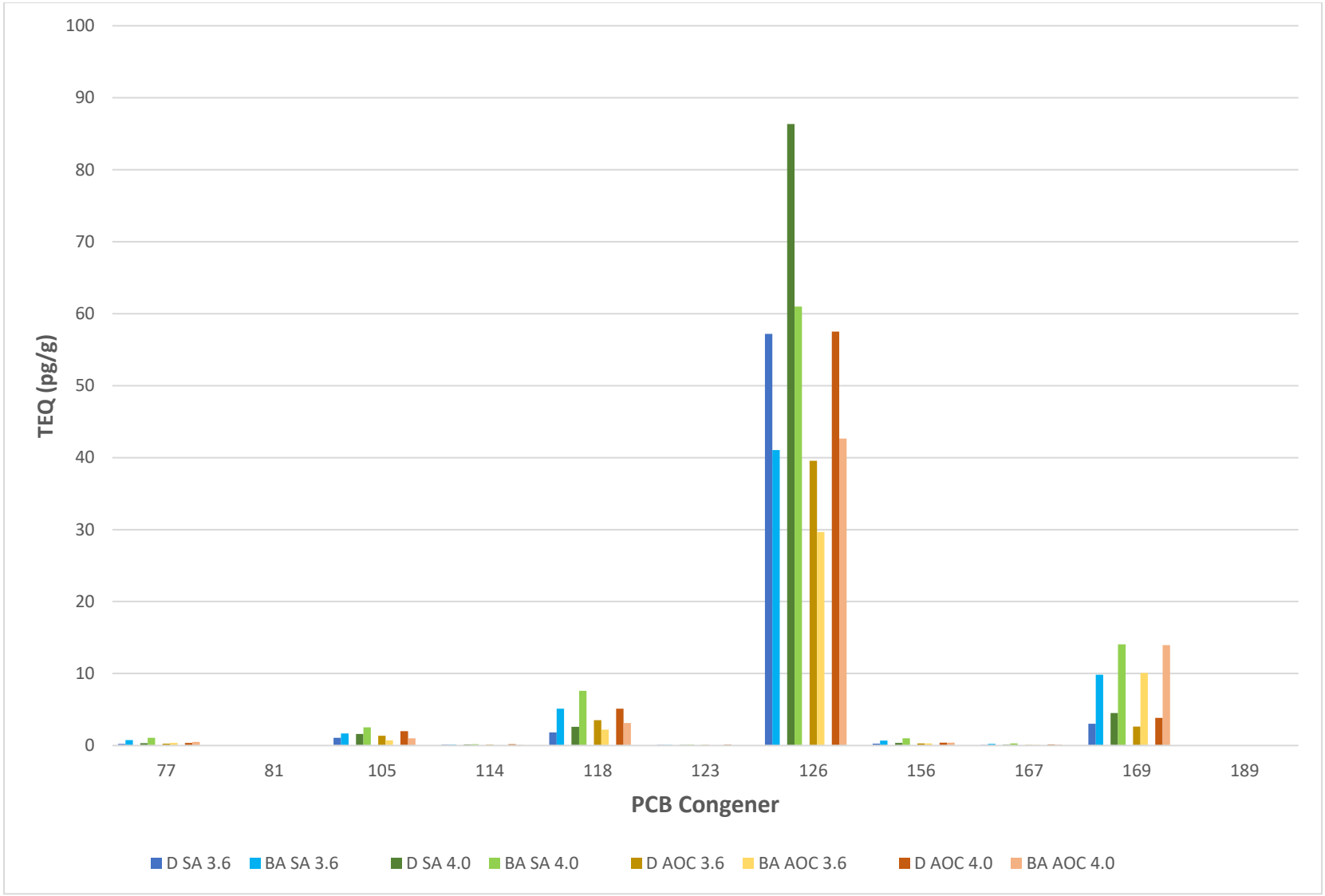
Appendix C: PCB Congener and PCB TEQ Modeling Results (next 2 pages).

Appendix C1. Concentrations of PCB congeners predicted by diet and bioaccumulation models.
Legend: D = Diet model, BA = Bioaccumulation model, SA = source area, AOC = area of concern, 3.6 and 4.0 are trophic levels.



Appendix C2. TEQ from PCB congeners predicted by diet and bioaccumulation models.

Legend: D = Diet model, BA = Bioaccumulation model, SA = source area, AOC = area of concern, 3.6 and 4.0 are trophic levels.



Appendix 4 – Public Outreach Summary