



Macroinvertebrate Diversity of Submerged Detroit River Coastal Wetlands

Jessica Robson¹  · Kenneth G. Drouillard¹ 

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Abstract

Urban rivers face sustained anthropogenic pressures limiting biodiversity. Yet, urban waterways such as the Detroit River are important habitat in supporting regional diversity. The Detroit River is a Great Lakes Area of Concern where conservation and restoration efforts prioritize improved biological and habitat integrity in the connecting channel. This study explores benthic macroinvertebrate in submerged aquatic vegetation across five mainstem channel wetlands and two tributary sites of the Canadian wetlands to describe spatial patterns and diversity. We first examine inter-wetland differences between five mainstem wetlands by hierarchical cluster analysis, NMDS and PERMANOVA, identifying two mainstem groups: one comprising of two middle reach wetlands (Detroit River Marshes and Grass Island), the second showed similarities among wetlands across all reaches (Turkey Creek, River Canard and Peche Island). The latter groupings shared similar habitat characteristics, deeper and finer grain-sizes, and functional feeding group characteristics - low abundances of shredders. Second objective, we perform an intra-wetland comparison for Turkey Creek and River Canard to analyze for differences along tributaries. At neither River Canard nor Turkey Creek we observed significant tributary influence on mainstem communities but had found the Turkey Creek tributary communities significantly differed from the channel communities. Diversity metrics and Hilsenhoff Biotic Index illustrate strained benthic communities across the river. We had also found water quality to be consistently moderately degraded. Our findings differ from prior analyses within emergent vegetation that indicate variable water quality conditions between mainstem and tributary and non-impaired macroinvertebrate communities.

Keywords Invertebrate · Submerged aquatic vegetation · Great Lakes · Tributary · Urban · Agricultural

Introduction

The Laurentian Great Lakes supports a growing 34 million people and are a biodiversity hot spot for 2200 aquatic taxa where 1433 are benthic invertebrates (Trebitz et al. 2019; Fergen et al. 2022). Benthic macroinvertebrates strongly influence nutrient and energy transfers from sediment to the water column and higher trophic organisms contributing to benthic-pelagic coupling (Grimm 1988; Wallace and Webster 1996; Demars et al. 2021). Anthropogenic stressors alter benthic community structures by altering hydraulic regimes,

physical substrate characteristics (e.g., siltation of formerly granular substrates), altering oxygenation status and oxygen penetration within sediments and contribute to toxicity via accumulation of toxic chemicals in sediment (Buss et al. 2004; Larsen et al. 2011; Scavia et al. 2014; Desrosiers et al. 2018). Many of the above stressors contribute to chronic effects such as reduced growth and reproduction and acute effects such as mortality leading to loss of sensitive species (reduced biodiversity) and in some cases proliferation of a limited number of tolerant species (reduced species evenness) (Evans-White et al. 2009; Ligeiro et al. 2013; Barnum et al. 2017).

Benthic macroinvertebrates are used in biomonitoring and bioassessments as environmental tolerances have been extensively studied and developed into indices (Resh et al. 1995; Reynoldson et al. 1995; Townsend et al. 1997) and established to respond to water and sediment quality conditions (Lock and Goethals 2011; Beermann et al. 2018;

✉ Jessica Robson
robso111@uwindsor.ca

¹ Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, ON N9B 3P4, Canada

Akyildiz and Duran 2021). The Hilsenhoff Biotic Index (HBI), developed in streams and used in river systems, estimates water quality and degree of organic pollution quantitatively utilizing macroinvertebrate taxonomic data (Hilsenhoff 1998; Gao et al. 2023). A common indicator group, Ephemeroptera, Plecoptera, and Trichoptera (EPT), are sensitive to contaminants, changes in organic loads, nutrients, and biological oxygen demand. The exclusion of sensitive species alters communities toward more tolerant ones (Chun et al. 2017; Saari et al. 2018). Pollution tolerant species are less desirable in supporting aquatic food webs due to bioaccumulation in tissues and low net energy (Bendell-Young 1999; Marcarelli et al. 2011; Bertoli et al. 2021; Das et al. 2023). Although invertebrates have been demonstrated to respond to disturbances, it can be difficult to assess highly impacted sites where diversity and indicator species are low (Ostermiller and Hawkins 2004). Species traits, such as functional feeding groups (FFGs) are available to gain further insights into functioning and the processing of energy in aquatic ecosystems (Cummins et al. 2005; Merritt et al. 2017). Several studies demonstrated relationships with FFGs responses to environmental perturbations. Typically, collectors (filters and gatherers) are pollution-tolerant whereas shredders and scrapers demonstrate environmental sensitivities (Carlisle and Clements 2005; Pastorino et al. 2020; Yaagoubi et al. 2023). Diversity-based tools involve niche partitioning as a central concept – the use of assigning taxonomic units to environmental gradients. Metacommunity analysis seeks to broaden niche-based approaches to consider traits, such as dispersal; regional trait databases are in development and providing insights into community structuring for management (Poff et al. 2006; Schmidt-Kloiber and Hering 2015; Sarremejane et al. 2020; Ao et al. 2022).

Species traits such as mobility (drift, crawling, swimming, flight) and species interactions (competition and predation) also affects community compositions (Mackay 1992; Kelly et al. 2006; Bonada et al. 2007). Community assemblages are influenced by habitat characteristics such as water depth, velocity, temperature, food sources, flow rates and substrate (Mackay 1992; Collier 1995; Buss et al. 2004; Jonsson et al. 2017; Demi et al. 2019). Many studies have analyzed community composition, particularly the Laurentian Great Lakes, yet studies on connecting channels are limited (Uzarski et al. 2017; Wick et al. 2019). These channels have a significant impact on biodiversity and consequences for management in the Great Lakes region by providing habitat and lake linkages for native and introduced species (Tucker et al. 2020). Connecting channels can have simplified habitats relative to multi-order streams and are characterized by high flow rates contributed almost entirely by the upstream water body along with minor water inputs stemming from

smaller tributary inputs. Habitat complexity is contributed by depth transitions around navigational channels, islands, and shoreline features such as small embayments and/or areas receiving tributary inputs that support submerged wetlands. Our study examines diversity and functional feeding groups of submerged Detroit River wetlands (Canada) to gain insight into community patterns in this Great Lakes connecting channel and Great Lakes Area of Concern to determine whether tributary-associated wetlands support different communities compared to wetlands present in the main channel and embayments.

The Detroit River receives >98% of its flow from the upstream Lake St. Clair. Water quality tends to remain consistent along most Canadian reaches before entering Lake Erie. Furthermore, deep, cooler, high velocity waters flowing through navigation channels throughout the river length have, for the most part, maintained hydraulic separation between nearshore water masses along Canadian and U.S. shorelines reducing the impact of U.S. legacy and point sources inputs on Canadian nearshore habitats (Drouillard et al. 2006). Sediment contamination in Canadian jurisdiction have been declining and are at suitable thresholds for benthos (Szalinska et al. 2013; DRCC 2020). However, there remain some concerns about localized impacts to water quality and potential implications to ecosystem indicators in Canadian nearshore areas subject to inputs from tributaries receiving urban and agricultural stressors. As tributaries join into rivers, areas of confluence can create physiochemical gradients depending on flow characteristics. These areas can increase biodiversity by creating more heterogeneity in a habitat. However, in stressed habitats benthic communities may be negatively impacted (Hayward et al. 2022).

Environment and Climate Change Canada (ECCC) routinely conducts biotic and water quality assessments at Canadian wetlands present in the upper, middle and lower reaches of the channel and two major tributaries, River Canard and Turkey Creek (ECCC 2022). Water quality ranges from good to severely degraded, with the two tributaries being moderately or severely degraded according to ECCC's water quality index (Chow-Fraser 2006; ECCC 2022). Despite poor water quality index rankings, assessment of macroinvertebrate integrity at Turkey Creek and River Canard tributaries did not indicate marked impairment relative to other monitored Detroit River wetlands within the channel. However, ECCC's surveys of macroinvertebrate communities are restricted to areas of emergent vegetation close to the shoreline (ECCC 2022). The submerged aquatic vegetation (SAV) sections that form large continuous beds connected with these marshes are non-wadable depths, located within the mainstem - upstream and downstream of the tributary discharge points. In this study, mainstem SAV beds adjacent to the tributary are referred to

as mainstem-tributary (MT) wetlands, at Turkey Creek and River Canard. Non-tributary (NT) submerged wetland beds are Peche Island, Grass Island and Detroit River Marshes (DRM).

We conducted a community survey of Detroit River macroinvertebrates with two main objectives. First, is to examine whether five mainstem wetlands vary in composition such that mainstem-tributary wetlands differ most from non-tributary wetlands. It is predicted that mainstem-tributary wetlands will differ as these areas receive inputs from tributaries with longstanding water quality issues and can cause changes in habitat (Svendsen et al. 2009). The second objective is concentrated on the mainstem-tributary wetlands, and to investigate whether sampling nearby tributaries is representative of channel communities in submerged vegetation. Wetlands were delineated upstream and downstream sections to test for community differences to tributary communities. We predict that given longstanding water quality differences between mainstem and tributaries, communities will vary between the tributary and adjacent mainstem communities. Additionally, components of community diversity are explored to describe diversity found within the Great Lakes Area of Concern.

Methods

Site Description

The Detroit River is a binational connecting channel in the Laurentian Great Lakes passing between the cities of Detroit, Michigan, USA and Windsor, Ontario, Canada. This connecting channel is also known as *Waawiatanong Ziibi* in Anishinaabemowin, within the Traditional Territory of the Three Fires Confederacy of First Nations (Ojibwe, Odawa, and Potawatomi). The watershed drains 19,040 km² through 49% agricultural land and 21% urban land where Canadian landmass represents 79% of agricultural land and 17% of the urban (Scavia et al. 2019). Water levels are dependent on Lake St. Clair and Lake Erie levels as the river has a gradual slope. It flows southward 43.5 km from Lake St. Clair to Lake Erie with a discharge rate ranging from 4400 m³s⁻¹ in the winter to 5,700 m³s⁻¹ in the summer (Derecki 1984). Sediment grain-size varies across the river as flow rates vary with changing depth, presence of islands and proximity to shorelines. Fine sediment (sand and silt) is common in the upper and middle reaches and very fine sediments (silt and clay) deposit mainly in the lower reach and in narrow canals. (Fallon and Horvath 1985; Wood 2004; Szalinska et al. 2013). The Canadian wetlands sampled within the Detroit River are Peche Island Marsh located in the upper reach, middle reach wetlands include Turkey Creek, Detroit

River Marshes (DRM), and Grass Island and River Canard found in the lower reach (Fig. 1). Two tributary inlets were sampled at River Canard and Turkey Creek.

Sampling Design

Each wetland was sampled over the period of September 24th – October 3rd, 2019. Wetland boundaries were determined with the aid of the Essex Region Conservation Authority On-Line interactive mapping tool (<http://ercamaps.countyofessex.ca/>) using the Provincially Significant Wetland layer tool and Google Earth Pro. A total of 60 sites were sampled with 10 sites across mainstem wetlands ($n=5$) and 5 sites per tributary ($n=2$). Sample locations were assigned using a stratified random sampling design. Each mainstem wetland was overlaid with a grid (~150 × 150 m) and sample locations assigned as the centre coordinate of 10 randomly selected grid cells. Mainstem-tributary wetlands were divided into 3 strata; two within the channel encompassing macrophyte bed positions upstream of the tributary input, the second strata downstream and the third within the tributary plume, each strata were sampled in five locations. These strata were delineated using Google Earth imagery and examining tributary plume extent dimensions across time using the historical imagery tool. Detailed maps of sample site locations at each of the five wetlands and tributaries are provided in Supplementary Information (Figures S1-S4).

Data Collection

Water quality data were collected using a Ruskin RBR profiler at each sample site to measure depth, conductivity, pH, temperature, and turbidity just above the sediment. Water quality was calculated as per Chow-Fraser (2006) according to the equation:

$$WQI = (-1.36 \times \log \text{Turbidity}) - (1.57 \times \log \text{Conductivity}) - (1.62 \times \log \text{Temperature}) - (2.37 \times \log \text{pH}) + 9.26$$

The associated qualitative descriptor has WQI values 0 to 1 as Good, 0 to -1 moderately degraded and <-1 as degraded (Chow-Fraser 2006). Bulk sediment for characterization was collected by petite ponar grab sampler for grain size distributions and loss on ignition derived sediment organic carbon content, referred to as Total Organic Carbon (TOC) in this study (Drouillard et al. 2006). Benthic macroinvertebrates were retrieved using triplicate grabs by petite ponar grab sampler (2.4 L), ensuring each accepted replicate came from a ponar grab that was at least ¾ full of sediment. Each sediment grab was washed on site through a 250 µm mesh and the sample preserved in a modified Kahles solution



Fig. 1 Study area of the Detroit River with Peche Island, Turkey Creek, Grass Island, Detroit River Marshes and River Canard wetland boundaries

(10:5:1 v/v/v water:95% ethanol: formalin). One of the three samples were randomly selected for in-lab macroinvertebrate identification by dissection microscope.

Data Analyses

Differences between habitats were investigated via pairwise-PERMANOVA (Euclidean distance, Bonferroni-corrected) on depth and sediment characteristics (grain-sizes, and TOC). Forty-one benthic macroinvertebrate taxa were identified from 14,694 observed individuals mostly to genus or species with exception of Oligochaeta, Chironomidae, Nematoda, Risssooidea, and Turbellaria by the taxonomic keys: Freshwater Invertebrates: Keys to Nearctic Fauna (Thorp and Rogers 2016); An Introduction to the Aquatic Insects of North America (Merritt et al. 2008). Biodiversity surveys are limited by sampling efforts to capture very rare species (Cao et al. 2001) and this study was limited in its sampling effort on a per wetland basis to interpret differences in rare species abundances across wetlands. To reduce noise in the dataset (Gauch 1982), rare taxa (occurrence in <10% of samples) were removed or where possible rare genera were combined to family level into a total of 28 taxa. This resulted in the removal of 26 individuals from 8 taxa that represented less than 3% of the relative abundance at each sample site (Resh et al. 2005). Individual species data was transformed to meet the assumptions of normality by an octave transformation of relative abundances (Preston 1948; Corkum et al. 1997).

Statistical analyses were performed by R-statistical software using *vegan* and *adespatial* packages (Oksanen et al. 2022, Dray et al. 2022), figures were generated in *ggplot2* and *heatmap* (Wickham 2016, Kolde 2019). Diversity metrics were calculated for each wetland and tributary for taxa richness, Pielou J's evenness, EPT taxa (%), and Sorenson beta-diversity of nestedness and turnover. Beta-diversity was calculated by *beta.div.comp* function within the R *adespatial* package using default settings for relative abundance data (Legendre 2014). Benthic macroinvertebrate community data were first examined using heat maps accompanied with cluster analysis of a Bray-Curtis community dissimilarity matrix by agglomerative hierarchical clustering (*stats* package; function *hclust*), Ward's method (*Ward.D2*) (Murtagh and Legendre 2014). Determining the number of optimal clusters was aided by *NbClust* (package and function) in R which provides results from 30 indices. While cubic clustering criterion supports two major clusters (Value Index: 7.2175), the weight of evidence supports three significant clusters by 9 indices value index: KL (4.022), Hartigan (2.4544), TrCovW (10596.97), Trace W (217.8027), Rubin (-0.1707), Silhouette (0.1697), Beale (-0.8409) and Ball (1036.522).

Community differences by wetland were visualized with non-metric multidimensional scaling (NMDS) ordination. PERMANOVA analyses with Bonferroni-corrected pairwise comparisons were then used to evaluate for community differences between the 5 mainstem wetlands (Bray-Curtis distances). We hypothesized that one or both mainstem tributary wetlands would deviate in their community structure from the 3 non-tributary wetlands. Next, the mainstem-tributary wetlands were examined independently to test for community differences with respect to tributary position (upstream, downstream, and tributary). Similar to mainstem-wetland contrasts, an NMDS ordination and PERMANOVA was performed to visualize and test for differences by position.

Hilsenhoff Biotic Index (HBI) values and FFGs were assigned under the following FFG classifications: collector-gatherer, collector-filterer, predator, parasite, scraper, and shredder. HBI Values and FFG designations were determined (Bode et al. 1996, 2002; Barbour et al. 1999). Significant differences among FFG relative abundances between wetlands and tributaries and differences were explored via PERMANOVA. For all PERMANOVA analysis on community composition and FFGs that bore significant differences, SIMPER analysis was performed using PAST Statistical Software (v. 4.03) with Bray-Curtis distances to provide insight into taxon influence on variation between communities (Clarke 1993; Hammer et al. 2001).

EPT taxa, *Hyallolella spp.*, and FFG count data associated with physiochemical properties of water quality and habitat characteristics using generalized linear models (GLMs). The distribution of the continuous data was non-normal (right-skewed), zero-inflated and overdispersed and not suitable for Gaussian or Poisson distribution – a gamma distribution hurdle model was utilized in this work as directed by Zuur & Ieno (2016). The hurdle model is comprised of two components, a Gamma GLM and Bernoulli GLM. In base R, presence/absence data was generated for the response variables for the binomial adjustment GLM (Bernoulli). The log link gamma model had response variables with zero entries removed which limits interpretation and sample size. Spearman rank-correlations coefficients were between the explanatory variables were assessed for values less than 0.50 to account for collinearity and minimize overfitting. In choosing the best model, the lowest Akaike Information Criterion (AIC) was selected. Models were generated for 13 taxa and 6 FFGs with 5 explanatory variable combinations. Models that had demonstrated significant associations were further analysed for the adjusted R^2 (Tjur or Nagelkerke) and models were cross-validated with *glmnet::cv.glmnet* function (Friedman et al. 2010) and mean squared error from subsetted training data. Relevant residual vs. fitted plots are provided as Figure S5.

Results Section

Environment

Mainstem-tributary sample sites were generally deeper with slightly higher conductivity and turbidity than non-tributary sample sites. Non-tributary wetland sites exhibited coarser grain sizes (Table S1). Mainstem-tributary wetlands, River Canard, the downstream positions were deeper with higher proportions of sand and reduced turbidity, TOC, and gravel (Table S2). Next, Turkey Creek's downstream had lower turbidity and depth with similar grain distributions, while the tributary had overall higher conductivity, TOC, and was the shallowest of the three positions (Table S2). PERMANOVA on habitat characteristics between wetlands yielded differences ($F_{4,44} = 4.67, p = 0.001$), pairwise analysis revealed differences between Grass Island (NT) to Peche Island (NT) ($F_{1,19} = 3.45, p = 0.030$), Turkey Creek (MT) ($F_{1,19} = 14.64, p = 0.002$), and with River Canard (MT) ($F_{1,19} = 6.80, p = 0.016$). All wetlands and tributaries in the present study are considered moderately degraded with scores below zero, ranked in descending quality: Grass Island (-0.16), DRM (-0.35), Peche Island (-0.39) & Turkey Creek (-0.39), River Canard (MT) (-0.47), River Canard Tributary (-0.5), and Turkey Creek Tributary (-0.71).

Macroinvertebrate Community

Across sampled wetlands, twenty-eight taxa were identified from 14,694 individuals. Final taxonomic resolution belonged to 2 species, 10 genus, 13 families, 1 sub-class (Oligochaeta), 1 class (Turbellaria) and 1 phylum (Nematoda). The most common taxa observed were Chironomidae and Oligochaeta at 100% of sites, Nematoda (95%), Risssoidea (86.6%), Valvatidae (83.3%) and Dreissenidae (73.3%).

Community diversity metrics are summarized in Tables 1 and taxon frequency tables by wetland are provided in Supplement 2. Across all mainstem wetlands ($n = 50$), Shannon H' was 1.764 from an average taxa richness of 13.2. Pollution sensitive, EPT taxa ($n = 8$) made up 7.17% of individuals and were found at 60% of sample locations. Beta-diversity between Detroit River as a whole was primarily taxa turnover (0.736). Mainstem-tributary wetland sample sites ($n = 20$) were dominant in Chironomidae (0.403 ± 0.168), Oligochaeta (0.219 ± 0.094), Nematoda (0.126 ± 0.109) and Risssoidea (0.087 ± 0.092). Alpha diversity metrics show taxa richness was 11.5, moderately even 0.653 (Pielou J), Shannon diversity was poor (1.584) with EPT taxa in low abundance (3.7%). Beta-diversity was predominately represented by taxa turnover (67.3%).

Non-tributary sites ($n = 30$) were similarly dominant in Chironomidae (0.315 ± 0.022), Oligochaeta (0.228 ± 0.023) and Nematoda (0.076 ± 0.009), followed by a Trichopteran, *Leptocerus spp.* (0.061 ± 0.014). Taxa richness was higher (14.4) than MT wetlands (11.5), and assemblages were slightly more even (0.715), negligibly more favourable Shannon Diversity, 1.88, with higher EPT presence of 10.6%. Turnover contributed to 71.5% of beta diversity. Three taxa were unique to non-tributary wetlands, the platyhelminth Turbellaria; the sole Odonate taxa detected, Coenagrionidae; and the Trichopteran *Nectopsyche spp.*

Hilsenhoff Biotic Index scores below 6.50 are considered to have fair water quality, and the Detroit River and tributaries scored fairly poor (6.69) suggesting significant pollution impairing communities (Hilsenhoff 1987). HBI scores in descending quality are Turkey Creek (MT) (6.550), River Canard Tributary (6.610), DRM (6.619), River Canard (MT) (6.690), Turkey Creek Tributary (6.73), Grass Island (6.735), Peche Island (6.905). Functional feeding groups were dominant in collector-gatherers, followed by scrapers and parasites. Scrapers were found in low abundances at

Table 1 Diversity measurements by site groupings

	Shannon H'	Richness (n)	Pielou J	EPT%	Turnover	Nestedness
Detroit River ($n = 50$)	1.764	13.22	0.69	7.17	0.736	0.264
Mainstem Tributary ($n = 20$)	1.584	11.5	0.653	3.746	0.755	0.245
Non-Tributary Mainstem ($n = 30$)	1.885	14.367	0.715	10.595	0.755	0.245
Detroit River Marshes (DRM) ($n = 10$)	1.843	14.9	0.69	14.509	0.652	0.348
Grass Island ($n = 10$)	1.849	15.2	0.683	12.868	0.736	0.264
Peche Island ($n = 10$)	1.962	13	0.773	4.408	0.713	0.287
River Canard (RC) ($n = 10$)	1.625	11.8	0.663	5.61	0.808	0.192
Turkey Creek (TC) ($n = 10$)	1.543	11.2	0.643	1.883	0.723	0.277
RC – Tributary ($n = 5$)	1.49	9.8	0.745	7.57	0.926	0.074
RC – Downstream ($n = 5$)	1.611	11.8	0.658	3.822	0.428	0.572
RC – Upstream ($n = 5$)	1.64	11.8	0.668	7.398	0.715	0.285
TC – Tributary ($n = 5$)	2.09	16.4	0.75	11.271	0.712	0.288
TC – Downstream ($n = 5$)	1.639	11.6	0.669	2.545	0.735	0.265
TC – Upstream ($n = 5$)	1.447	10.8	0.617	1.221	0.735	0.265

Peche Island (NT), River Canard (MT), and Turkey Creek (MT) (Fig. 2). Significant differences among wetland FFGs were found with Grass Island showing differences to Peche Island ($F_{6,53} = 10.85$, $p=0.017$), Turkey Creek – mainstem ($F_{1,19} = 3.261$, $p=0.001$) and tributary ($F_{1,14} = 6.48$, $p=0.0273$). SIMPER indicates differences driven by collector-gatherers and shredders (Supplement 3).

Variable selection by Spearman correlation analysis reduced environmental variables to conductivity, turbidity, depth, TOC, silt for GLM models (Table S3). Nine models displayed significant values, however in cross-validation, mean standard error derived from training and test data were high for all but two presence/absence models for *Caenis spp.* and *Hyaella spp.* indicating depth as a significant predictor of their establishment. For the genus *Caenis*, regression considered depth, TOC and silt, the adjusted R^2 value was reported to be weak at 0.11 and the AIC value was equal to 75.6. The effect of depth was statistically significant and negative (beta = -1.39, 95% CI [-2.91, -0.14], $p=0.047$; Std. beta = -0.72, 95% CI [-1.18, 0.18]). The *Hyaella spp.* model considered conductivity and depth with a moderate Tjur's R^2 (0.21). The effect of depth is statistically significant and negative (beta = -2.40, 95% CI [-4.04, -1.03], $p=0.002$; Std. beta = -1.24, 95% CI [-2.09, -0.53]). Table of regression parameters for all 9 models are provided in Table S4.

Between Wetland Benthic Community Contrasts

Cluster analysis of benthic communities by wetland shows there were three major clusters of communities, (Fig. 3, Figure S6). The first cluster indicates similarity between Grass Island (NT), DRM (NT), and Turkey Creek Tributary. The following two clusters stem from the same branch, one showing similarities between Peche Island (NT) and Turkey Creek (MT), and the second is predominately River Canard sites (MT and Tributary). River Canard mainstem sites are distributed among both clusters. Similar observations were generated according to the NMDS ordinations. In order to achieve an acceptable stress threshold (<0.2) (Clarke 1993), the NMDS ordination was completed across four dimensions (stress: 0.134), Fig. 4 presents the ordination across the first 2 NMDS axes. 2-D plots across the remaining axes are provided in Supplementary (Figures S7-S11). Turkey Creek communities demonstrated moderate variability with sites resembling communities found at Peche Island whereas River Canard shown greater community variance with similarity to all wetland communities across NMDS1-NMDS2 (Fig. 4).

PERMANOVA indicated significant differences in macroinvertebrate community composition between the wetlands ($F_{4,45} = 5.296$, $p=0.001$). Pairwise-comparisons (Bonferroni-corrected p -values) indicated Peche Island resembled Turkey Creek ($p=0.074$) and River Canard ($p=0.056$). All remaining between wetland comparisons were significantly different ($p<0.05$) from one another. PERMANOVA and

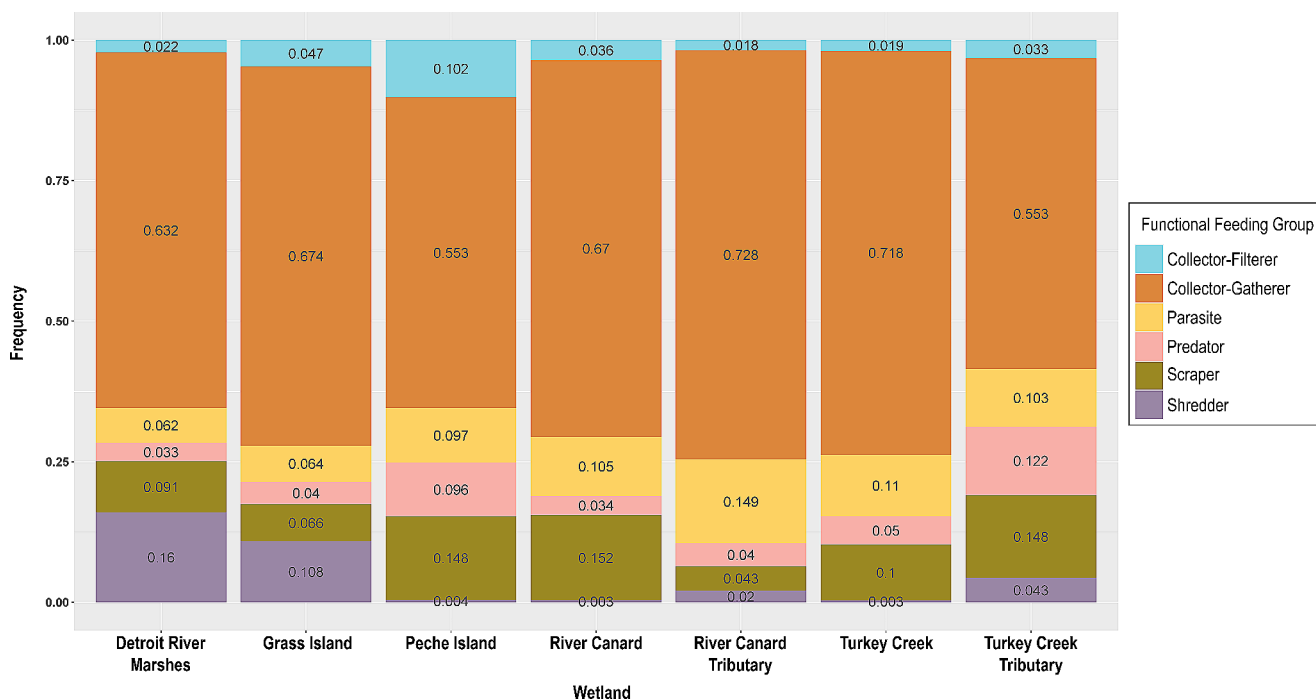


Fig. 2 Functional feeding groups of mainstem and tributary wetland sites in Detroit River SAV beds

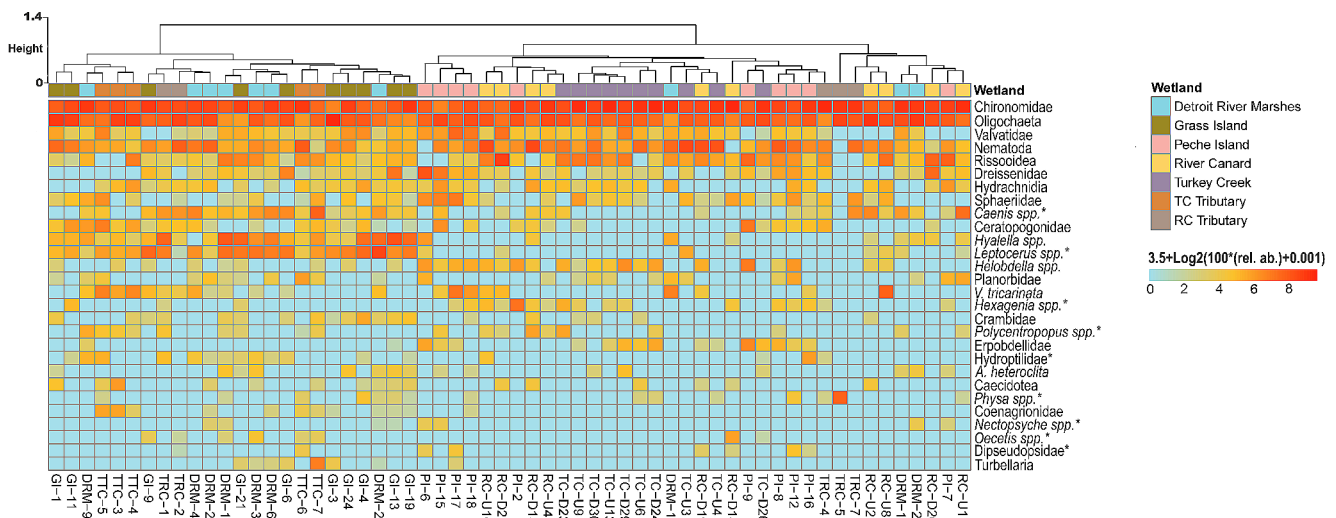


Fig. 3 Heat map with cluster analysis of five mainstem wetland and two tributary communities annotated with wetland designation. (See Figure S6 for enlarged cluster dendrogram) (*denotes EPT taxa)

SIMPER results for all significantly different wetland pairs are provided in Supplement 3. It should be noted that the assumption for homogeneity of multivariate dispersions was not valid for the contrast between River Canard compared to DRM, Grass Island, and Turkey Creek. Therefore, River Canard taxonomically can only be compared to Peche Island with confidence by PERMANOVA (Supplement 3).

Between Strata Differences in Macroinvertebrate Communities at River Canard

Sample sites in the upstream and downstream strata of River Canard had similar taxa richness (11.8) and Pielou J's evenness (0.658 and 0.668) with greater abundances of EPT taxa found at upstream (7.40%) compared downstream strata (3.82%). The tributary plume strata had the lowest taxa richness (9.8), highest Pielou J's evenness 0.745, and EPT taxa comparable to upstream sites (7.57%). The most abundant upstream and tributary plume taxa were Chironomidae, Oligochaeta, Nematoda and *Caenis spp.* whereas the downstream was predominately Chironomidae, Oligochaeta, Risssoidea and Nematoda. Taxa turnover was lower in the downstream strata resulting in greater partitioning to nestedness at downstream (0.572) compared to upstream strata (0.285). Beta diversity at the tributary plume was predominately partitioned into taxa turnover (0.926) which was notably greater than upstream (0.715) and downstream (0.428) (Table 1). Richness within the tributary, sites TRC-5 and TRC-7 had the lowest taxa richness detected in this study. Three taxa were identified at TRC-05 (Chironomidae, Oligochaeta and *Physa spp.*) and five taxa were found at TRC-7 (Chironomidae, Oligochaeta, Nematoda, Sphaeriidae, and *Caenis spp.*).

NMDS ordination of benthic communities across River Canard strata ($k=3$, stress=0.061) showed a high degree of overlap in community ordination space (Fig. 5; Figures S12-S13). There were also no significant differences in macroinvertebrate community composition by taxa or FFGs by PERMANOVA ($p > 0.05$) detected across the three strata.

Between Strata Differences in Macroinvertebrate Communities at Turkey Creek

The most abundant taxa were similar across the three Turkey Creek strata and were dominated by Chironomidae, Oligochaeta, Nematoda, and Risssoidea. Diversity metrics at upstream and downstream strata demonstrated similarities with respects to richness (10.8 & 11.6), Pielou J's evenness (0.617, 0.669) and low abundances of EPT (1.22% and 2.55%). Sites within the tributary plume had higher richness (16.4) and Pielou J evenness (0.750), EPT% (11.28). Beta diversity did not markedly vary and was primarily partitioned into turnover (0.712–0.735).

NMDS ordination of community structure at Turkey Creek ($k=3$ stress=0.077) showed overlap between the upstream and downstream strata of Turkey Creek that were distinctly separated from tributary plume on NMDS1-NMDS2 (Fig. 5) and NMDS1-NMDS3 (Figure S14-S15). PERMANOVA also revealed significant differences between the strata ($F_{2,12} = 5.803, p=0.001$). Pairwise comparisons reaffirmed significant differences in the community structure between the tributary to upstream ($F_{1,3} = 7.744, p=0.024$) and tributary to downstream ($F_{1,3} = 8.14, p=0.027$) strata. Community composition in the upstream of Turkey Creek had the lowest Shannon H' in this study with similar richness (10.8) to the downstream (11.6). The

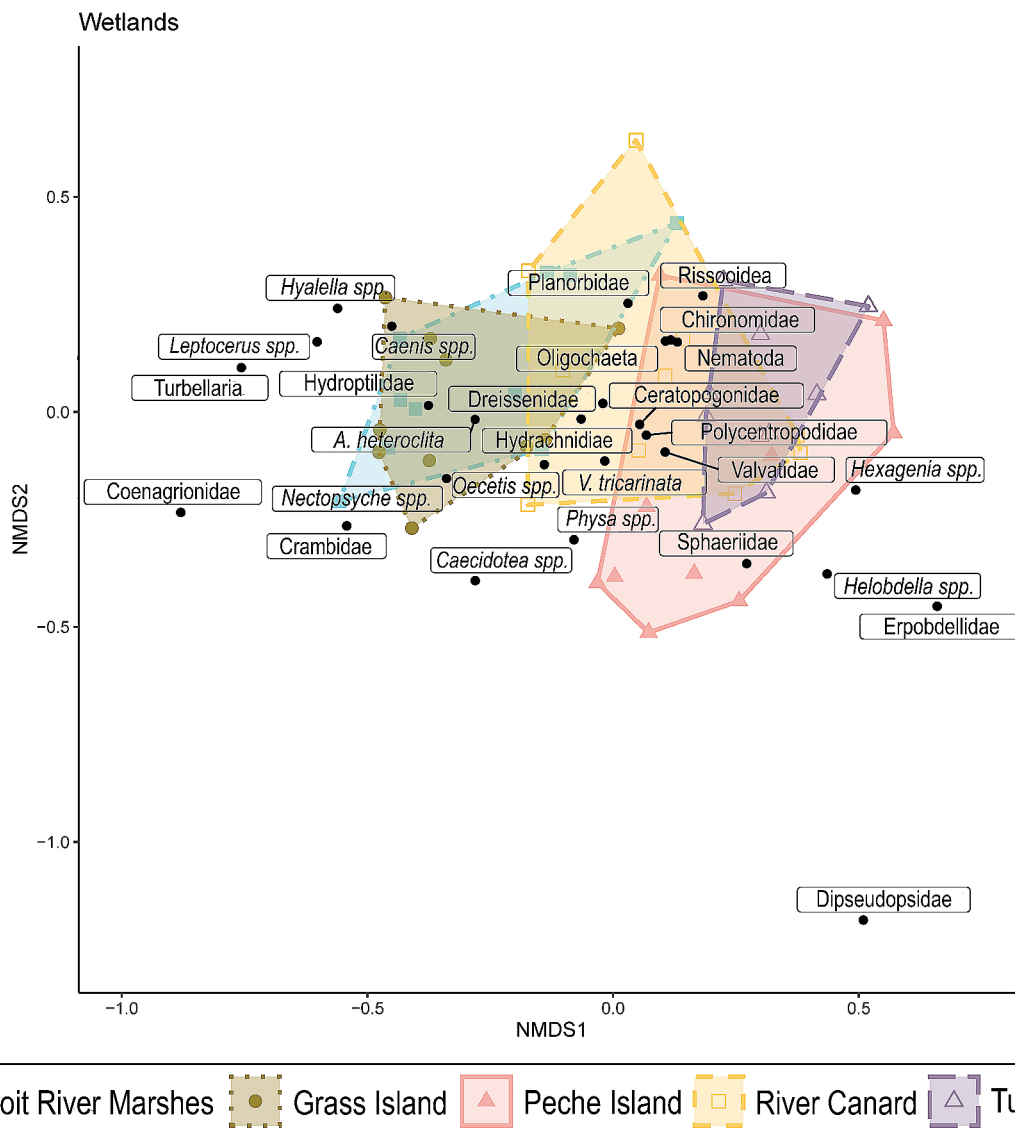


Fig. 4 NMDS ordination of five Detroit River mainstem wetland communities: benthic macroinvertebrate communities by wetland with species scores plotted on NMDS1-NMDS2 ($k=4$, stress=0.134)

lowest EPT%, 1.22, was in the upstream of this wetland. It was observed sites TC-U3 and TC-U4 in the upper margins had slightly reduced richness (8–9 taxa). With exception to *Leptocerus spp.* found at TC-U3, taxa at sites can be described as pollution tolerant taxa, Chironomidae, Oligochaeta, Nematoda, Erpobdellidae and six Mollusca taxa. SIMPER analysis on taxa between Turkey Creek upstream to tributary indicate differences are largely represented by increased diversity and abundances at the tributary. Similarly, Turkey Creek downstream to Turkey Creek tributary indicate several taxa excluded or in low abundances in the downstream with more pronounced *Helobdella spp.* (Hirudinea) in the downstream (Supplement 3).

Distributions of FFGs between Turkey Creek strata held weak differences (non-significant) between the upstream

and tributary ($F_{1,3} = 3.700$, $p = 0.08$) with Collector-Gatherers and Parasite groups carrying 53.11% of the dissimilarities (Supplement 3). The frequency of the collector-gatherer Chironomidae in the upstream portion was 0.493 and 0.263 in the tributary and the parasite, Nematoda, was also in higher abundances in the upstream, 0.166 to 0.066 in the tributary (Supplement 2).

Discussion

Our results generally supported the first hypothesis that there are significant differences in community composition across the mainstem wetlands. However, these differences did not conform to the predication that mainstem-tributary

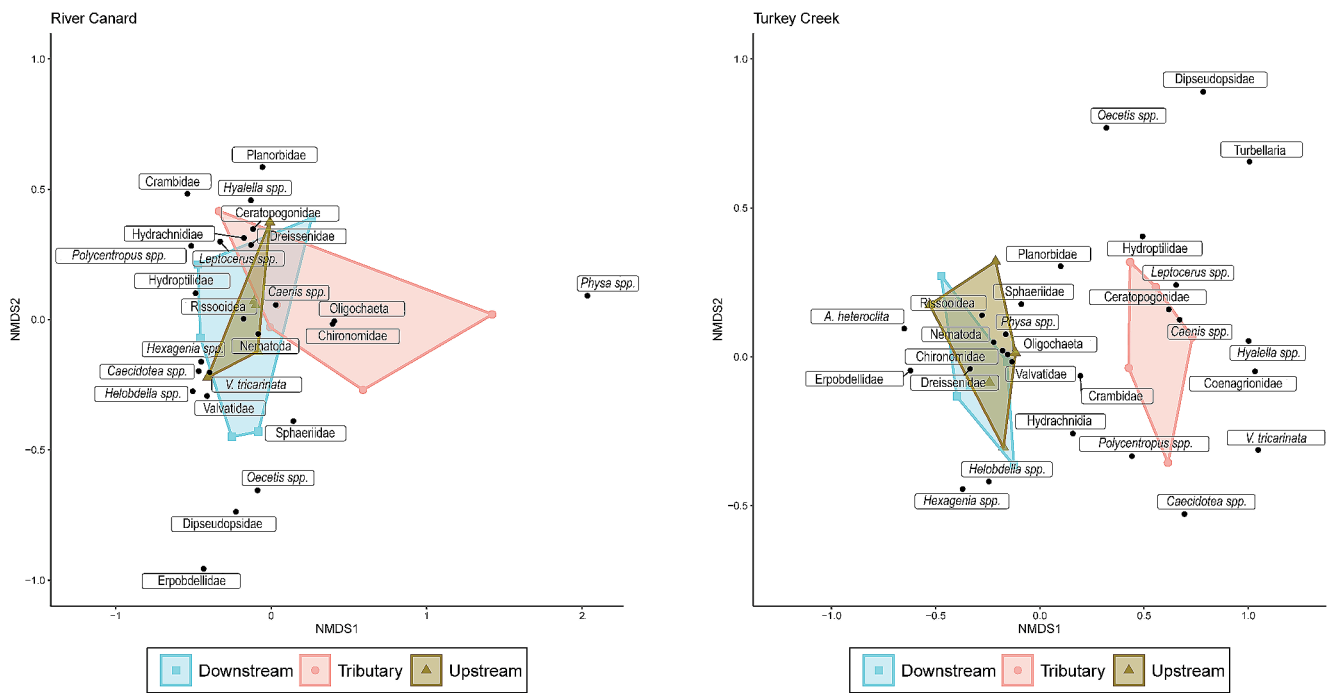


Fig. 5 NMDS ordinations of benthic macroinvertebrate communities of mainstem wetland (upstream and downstream) and tributary at River Canard ($k=3$, stress=0.061) (left) and Turkey Creek ($k=3$ str=0.077) (right) with species scores shown on NMDS1-NMDS2

wetlands differ most from the non-tributary wetlands and by extension differences were not observed between the upper reach (Peché Island), middle reach (DRM, Grass Island, and Turkey Creek) and the lower reach wetlands (River Canard). River Canard, Turkey Creek and Peché Island bore the most similarities by taxa, FFGs and lower diversity than DRM and Grass Island communities. This differs from Harris (1999) community analysis in the St. Clair River, a Great Lakes connecting channel, that observed changes in community along the first 10 km followed by a consistent 18 families per site across the length of the channel, whereas we had found variable richness throughout the channel, the average richness was lowest at mainstem-tributary wetlands and Peché (11–13) and highest at mid-reach mainstem wetlands, DRM, and Grass (15). Importantly, Harris found community compositions had changed across years, which highlights a limitation in this study - a single year survey. The second hypothesis seeking community variation between the tributary plume, upstream mainstem and downstream mainstem was rejected River Canard and partly supported at the Turkey Creek wetland. At Turkey Creek, there were no significant differences between the upstream and downstream mainstem. However, there were clear differences between mainstem strata and the tributary communities. The tributaries at Turkey Creek are providing conditions more suitable in supporting diverse macroinvertebrate communities. At this wetland location, sampling the wadable portions within emergent and submergent zones of the tributary does not

reflect communities within the mainstem. However, at River Canard communities within the tributary inlet were similar to the mainstem wetland communities.

In-situ water quality readings taken at the time of sample collections demonstrated no differences in water quality across mainstem wetlands. These differences did not correspond with expected patterns of water quality differences described by prior studies (ECCC 2022). Data from the present research showed all wetlands including tributaries to have WQI scores considered moderately degraded, whereas the ECCC data indicate large between-wetland differences in WQI ranging from good at NT wetlands (DRM, Grass Island, Peché Island) to degraded (Turkey Creek Tributary and River Canard Tributary).

The between-wetland study differences in water quality scores could be related to the timing of sampling (multiple years for ECCC and a single year for the present work), the channel may experience seasonal variation (Yang et al. 2021) but there were also differences in the spatial scope of sampling adopted by each study. In the present study, wetlands were defined by submerged macrophyte edge boundaries with all portions of the wetland bed (submerged and emergent vegetation areas in tributaries) having an equal chance of being sampled. In contrast, the ECCC approach applied a directed-sampling design with repeated sampling at specific locations taken across years. Importantly, the two studies differed in sampling coverage of each wetland. ECCC’s study focused on wadable portions of the wetland

near emergent vegetation that restricted sampling efforts in closer proximity to shorelines. The present research incorporated a larger potential sampling area at each wetland by including deeper sections of submerged macrophyte beds. For the mainstem-tributary wetlands, this enabled separate delineation and sampling of upstream and downstream strata surrounding the tributary plumes that would have been missed in the ECCC's sampling campaigns. Additionally, sediment retention in submerged aquatic vegetation can be poorer than emergent vegetation, contributing to lower scores found in our study (Gurnell and Bertoldi 2022).

Habitat characteristics produced some variation between mainstem wetlands, but many wetlands provided similar sediment characteristics and depth such as Peche (NT), River Canard (MT), and Turkey Creek (MT) ($p=1$) whereas DRM (NT) and Grass (NT) were similar to one another, being shallower with coarser sediments. Interestingly, community composition partly resembled these relationships. River Canard was most similar to Peche Island whereas Turkey Creek was only similar to Peche Island. Shredders, contributors of fine particulate organic matter, were found in low abundances at these three wetlands which are common for areas with riparian zone impacts (Barbour et al. 1992). SIMPER shown that differences found between Turkey Creek (MT) to DRM (NT) and Grass Island (NT) are in the absence or low abundance of collector-gatherers *Hyalella spp.*, *Caenis spp.* and the shredder *Leptocerus spp.* at Turkey Creek. *Hyalella spp.* absence or low abundance at Peche Island (NT), River Canard (MT), and Turkey Creek (MT) contributes to dissimilarities to DRM (NT), Grass Island (NT). Studies have shown low abundances of *Hyalella spp.* associated to agricultural land uses (Cooper et al. 2007; Altieri et al. 2022) and can be restricted to shallow depths (Limén et al. 2005). In the models generated in this study we found support for potential exclusion by depth for *Hyalella spp.* and *Caenis spp.* genera. Turkey Creek and Peche Island had pronounced abundances of Hirudinea taxa, *Helobdella spp.* (*H. Stagnalis*) and Erpobdellidae. Hirudinea can be indicators of stress due to their moderate tolerances and are regularly associated with polluted areas as *Helobdella stagnalis* demonstrates preference for areas with elevated organic nutrient pollution (Tavzes et al. 2006; Kazancı et al. 2015). In this study, the Peche Island community was not categorized as a tributary-impacted wetland. However, they bore more similarities in community composition, functional feeding groups and habitat properties than the remaining non-tributary wetlands. The wetland community at Peche is located at the opening of the Detroit River, nested within an island, and is upstream of major urban development. Prior studies monitoring Peche Island has curiously shown higher dissolved phosphorus concentrations than the upstream Lake St. Clair, such that there may

be a localized effect of elevated dissolved phosphorus from southeastern Lake St. Clair agricultural tributaries onto the sediments. (Burniston et al. 2009; Colborne et al. 2019; ECCC 2022). The stressors at Peche Island may be more representative of agricultural signals from the upstream waters but the stressor sources in the Detroit River are varied and difficult to distinguish (Maguire et al. 2019). Communities at River Canard (MT) demonstrated similarity to all wetlands in the NMDS ordination but had marked multivariate dispersion compared to the other sites suggesting caution in interpreting PERMANOVA results for this wetland. However, cluster analysis had revealed River Canard sites to resemble Peche Island (NT) and Turkey Creek (MT) communities. Provided the similarities between the three wetlands with respects to habitat, community compositions, functional feeding groups, we do not observe significant differences between the river's reaches so much as more localized variation across the river which supports differences between wetlands. This variation is likely influenced by species traits, stochastic processes, and slight differences in habitat characteristics, such as depth and grain-size. Tall et al. (2016) in a three-year study on macroinvertebrates in a St. Lawrence River Lake found that sites become more stable from wave energy as depths increase over a meter, a depth many of our sites had exceeded. They found water depth, water level change, and sediment characteristics (silts, nitrogen) to be significant influences on community compositions. The Great Lakes had experienced a period of high-water levels at the time of this study. Coastal wetlands in the Great Lakes were found to migrate closer to land with a decrease in inundation and extent, submerged beds had shown the least change (Anderson et al. 2023).

It is unlikely water quality conditions is further differentiating community composition, degraded water conditions were observed throughout the study, and Hilsenhoff Biotic Index demonstrates communities which are all under water quality stress and/or significant organic pollution. As an urban waterway, nutrients, metals, and organic pollutants are a long-term problem in the area despite significant remediation progress. As sediment contamination and water quality remains consistent, it is likely water and sediment quality is a consistent determinant of colonization capabilities in the connecting channel.

Tributaries have the potential to modify main channel communities with the introduction of sediments, nutrients, contaminants, and organic matter (Bruns et al. 1984; Kiffney et al. 2006; Wallis et al. 2009; Howell et al. 2012) or aid in dispersal between mainstems and tributaries as invertebrates can drift (Elliott 2003; Wilson and McTammany 2014). In seeking community variation with respects to upstream, downstream, or tributary position, we had not found significant differences between upstream and

downstream portions. Similar to Milner et al. (2019) we had not found increases in diversity downstream of tributaries in contrast with studies of communities around tributaries that found increased diversity downstream of tributaries (Vinson 2001; Katano et al. 2007). The effect of tributaries on rivers is influenced by channel and valley morphology such as basin shape, network patterns, and relative size of tributary and mainstem widths (Benda et al. 2004; Kiffney et al. 2006).

River Canard is an agricultural tributary with long-term water quality issues associated with nutrients, turbidity, and conductivity. Previous studies identified a high degree of extinction debt of fish communities at River Canard, with this location being among the most severe in the Lake Erie region (Montgomery et al. 2020). The River Canard downstream strata had the most nested beta-diversity. The elevated degree of downstream nestedness may be indicative of a pressure, natural or anthropogenic, in the area that is contributing to more homogenized macroinvertebrate community compositions. Nested communities are generally interpreted as the effects of a differential local extinction resulting in subsets of species whose tolerances, recolonization, and dispersal capacities are congruent with conditions at the habitat (Cutler 1994). The downstream locations were deeper which may have contributed to altered communities as depth is a significant factor to community compositions (Nelson and Steinman 2013; Schummer et al. 2021). Distance to agricultural tributaries commonly correlates with gradients of elevated conductivity, turbidity, and nitrates in wetlands (Schock et al. 2014). Two tributary locations were marked by reduced taxa richness of 3–5 taxa; TRC-05 had only three taxa (Chironomidae, Oligochaeta and *Physa* spp.) and TRC-07 had five taxa detected (Chironomidae, Oligochaeta, Nematoda, Sphaeriidae and *Caenis* spp.). *Physa* spp. are a scavenger/scrapper mollusc with preference for periphyton and benefit from elevated nutrients, (Stelzer and Lamberti 2001; Lombardo and Cooke 2002) characteristic of agricultural streams (Heathwaite et al. 1996). The mayfly, *Caenis* spp. is also a consumer of periphyton associated with good-to-moderately degraded habitats and are common to agricultural streams (Zumberge et al. 2003; Alhejoj et al. 2014). The remaining three tributary sample sites were located along nearshore plume zones of the tributary-mainstem junction and supported relatively diverse communities in terms of richness and EPT%. Near-shore conditions may provide more optimal conditions and communities than within the tributary inlet. However, more sample sites are needed to investigate unbalanced biodiversity at the tributary.

For Turkey Creek, the tributary plume had significantly different macroinvertebrate composition compared to benthic composition of the upstream and downstream strata of

this wetland. The tributary had the highest taxa richness, evenness, Shannon diversity (H'), and EPT% in this study. EPT taxa were in low abundance throughout the mainstem wetland (1.83% as a whole), EPT taxa in the upstream and downstream strata were 1.22% and 2.55%, respectively, compared to 11.27% in the tributary plume; the latter being comparable to the non-tributary impacted wetlands of the Detroit River. Overall, EPT% was low in this study (Qu et al. 2023), low prevalence of EPT taxon richness is expected for an urban tributary as they are prone to fluctuating hydrologic regimes and contaminated sediments which can reduce their richness (Konrad and Booth 2005). Yet, urbanized areas have a range of habitat types that can resemble natural areas in flow velocities and conditions supporting richness (Goertzen et al. 2022).

In contrast to the tributary, the upstream and downstream strata of Turkey Creek had taxa dominated by pollution-tolerant taxa including Chironomidae, Oligochaeta, Nematoda, and Risssooidea and were lacking or lower in relative abundances of sensitive taxa compared to the tributary strata. This suggests that benthic communities at the Turkey Creek wetland are compromised, but that the stressor does not appear to derive from the tributary input but rather from proximate stressor sources in immediately upstream portions of the Detroit River.

The most upstream portion of Turkey Creek is within 600 m of a salt-mine and 4 km of a wastewater treatment plant (WWTP). WWTPs can be a source of additional inorganic nutrients, metals, and other contaminants (Rosario-Ortiz et al. 2007; VanDrecht et al. 2009; Zamora-Ledezma et al. 2021). Mining activities involve altered topography by the removal of topsoil and vegetation and are areas of compacted soil due to industrial activity, which are all detrimental characteristics contributing to storm run-off (Negley and Eshleman 2006). Water quality measurements at Turkey Creek were broadly consistent with measured values at other wetland sites of the Detroit River. This however does not negate the possibility of periodic inputs related to storm events, where the effects of run off or wind-dispersal can impair water quality over short time scales (Chen and Chang 2019; Delpla et al. 2023).

Macroinvertebrate communities strongly associate with submerged macrophyte beds in terms of macrophyte species compositions, coverage, and by creating habitat complexity (Berg et al. 1997; Cheruvilil et al. 2002; Thorp et al. 2006; González-Ortiz et al. 2016). Agricultural and urban stressors both contribute nutrients, metals, and persistent organic pollutants (Maguire et al. 2019). These complex stressors can alter SAV macrophyte composition and abundance and shift FFGs in macroinvertebrate communities (Kolada 2010; Altieri et al. 2022). In our community survey, Odonates were not found at mainstem-tributary wetlands and a single

individual was found at Peche Island wetland. Vegetation cover and diversity in SAV beds can constrain Odonate populations strongly in urban landscapes due to fragmentation and human disturbances functioning as barriers (Watts et al. 2004; Sato et al. 2008). Jeanmougin et al. (2014), identified percent coverage of SAV as a primary driver in supporting robust Odonate diversity in urban ponds. A limitation in our study was not including an assessment of the integrity of SAV beds, which is commonly conducted in Great Lakes management toward restoration of fish habitat (Grabas et al. 2012; Midwood et al. 2021; ECCC 2022). However, Detroit River SAV beds are frequently qualitatively described as fair to good considering SAV species traits prioritizing water quality (turbidity tolerance, nutrient tolerances and algae) and percent coverage. These assessments find coverage often below 6% (ECCC 2022). Light attenuation in the Detroit River may be affecting SAV vegetation, best predicted by chlorophyll-a and turbidity measures (Scanell, 2023) which affects establishment depths and biomass (Hudon et al. 2000).

The quality of SAV habitat for aquatic organisms, such as fish, must reflect conditions suitable to support diverse macroinvertebrate communities. Our study describes community impairment within SAV beds despite good communities found in emergent vegetation in biomonitoring programs. Monitoring of SAV bed quality is an important step toward habitat restoration in the region for fisheries and macroinvertebrate communities improve with long-term management of wetlands (Schad et al. 2020). SAV beds are often overlooked sources of carbon pools because their contributions are usually outweighed by other carbon sources. In systems, such as the Detroit River, with highly modified shorelines, SAV beds can be an energy source to support aquatic systems (Paice et al. 2016).

In summary, we had not found significant differences in water quality from measurements taken above the sediment in the submerged aquatic beds such that all wetlands and tributary inlets were considered poor in water quality. However, we observed differences in benthic macroinvertebrate communities between these wetlands and determined the communities to all be impaired. Both findings are inconsistent with findings by environmental agencies which sample shallower emergent vegetation, in fewer sample sites, using sweep-netting methods across multiple years. With respects to the Detroit River's Area of Concern status in the Great Lakes, the Canadian benthic macroinvertebrates have been upgraded to non-impaired as sediment contamination had reached acceptable levels to support benthic communities and surveys indicate non-impaired communities in emergent vegetation. Quality fish habitat is an outstanding impairment within the Detroit River Area of Concern and submerged aquatic vegetation can provide critical habitat

for fish species to hide, spawn, and find food resources supporting diverse communities (Miller et al. 2018). Our study demonstrates that a food resource, benthic macroinvertebrate communities, as poor within submerged vegetation that are overlooked using current sampling protocols.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13157-024-01829-0>.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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