Science of the Total Environment 820 (2022) 153224

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Impacts of wastewater treatment plants on benthic macroinvertebrate communities in summer and winter

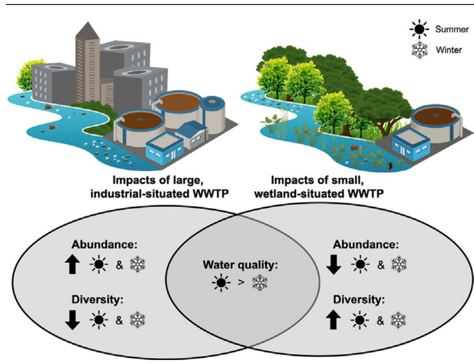
Chelsea Aristone [a,1], Hossein Mehdi [a,*,1], Jonathan Hamilton [a], Kelly L. Bowen [b], Warren J.S. Currie [b], Karen A. Kidd [c,d,e], Sigal Balshine [a]

[a] Department of Psychology, Neuroscience & Behaviour, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada
[b] Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, 867 Lakeshore Road, Burlington, ON L7S 1A1, Canada
[c] Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada
[d] School of Earth, Environment and Society, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada
[e] Institute for Water, Environment and Health, United Nations University, 204 – 175 Longwood Road S., Hamilton, ON L8P 0A1, Canada

HIGHLIGHTS

- Minor seasonal differences in biodiversity responses to wastewater
- Large, industrial-situated WWTP had higher abundance & lower diversity near outfall.
- Small, wetland-situated WWTP had lower abundance & higher diversity near outfall.
- WWTPs impaired water quality of downstream sites, especially in winter.
- Benthic communities closest and farthest from the outfall were the most dissimilar.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 October 2021
Received in revised form 12 January 2022
Accepted 13 January 2022
Available online 19 January 2022

Editor: Sergi Sabater

Keywords:
Seasonality
Sewage
Community ecology
Benthos
Biodiversity

ABSTRACT

Treated effluent from municipal wastewater treatment plants (WWTPs) is a major source of contamination that can impact population size, community structure, and biodiversity of aquatic organisms. However, because the majority of field research occurs during warmer periods of the year, the impacts of wastewater effluent on aquatic communities during winter has largely been neglected. In this study, we assessed the impacts of wastewater effluent on aquatic benthic macroinvertebrate (benthos) communities along the effluent gradients of two WWTPs discharging into Hamilton Harbour, Canada, during summer and winter using artificial substrates incubated for 8 weeks. At the larger of the two plants, benthic macroinvertebrate abundance was higher and diversity was lower at sites downstream of the outfall compared to upstream sites in both seasons. Whereas at the smaller plant, the opposite was observed, abundance increased and diversity decreased with distance from the outfall in both seasons. While the impacts of wastewater on benthic communities were largely similar between seasons, we did detect several general seasonal trends – family diversity of macroinvertebrates was lower during winter at both WWTPs and total abundance was also lower during winter, but only significantly so at the smaller WWTP. Further, benthic macroinvertebrate community composition differed significantly along the effluent gradients, with sites closest and farthest from the outfall being the most dissimilar. Our contrasting results between the WWTPs demonstrate that plants, with different treatment capabilities and effluent-receiving environments (industrial/urban versus wetland), can dictate how wastewater effluent impacts benthic macroinvertebrate communities.

* Corresponding author.
E-mail addresses: aristonc@mcmaster.ca (C. Aristone), mehdih1@mcmaster.ca (H. Mehdi), hamilj11@mcmaster.ca (J. Hamilton), kelly.bowen@dfo-mpo.gc.ca (K.L. Bowen), warren.currie@dfo-mpo.gc.ca (W.J.S. Currie), karenkidd@mcmaster.ca (K.A. Kidd), sigal@mcmaster.ca (S. Balshine).

[1] Denotes joint first authors with equal contributions.

http://dx.doi.org/10.1016/j.scitotenv.2022.153224

1. Introduction

Effluents discharged from municipal wastewater treatment plants (WWTPs) are one of the largest sources of aquatic pollution (by volume) in many parts of the world (Holeton et al., 2011; Hamdhani et al., 2020). Wastewater effluents, although treated in many jurisdictions, still contain a wide variety of contaminants beyond just phosphates and nitrogenous waste products, such as pharmaceuticals and personal care products (PPCPs), natural and synthetic hormones, micro- and macroplastics, agricultural and industrial chemicals, and metals (Daughton and Ternes, 1999; Kolpin et al., 2002; Ternes et al., 2004; Holeton et al., 2011; McCormick et al., 2016; Hamdhani et al., 2020). The continuous release of wastewater effluents into waterbodies subjects aquatic biota to chronic exposure of complex mixtures of contaminants, eutrophication, oxygen depletion, thermal pollution, and overall habitat degradation (Brown et al., 2011; Holeton et al., 2011; Tetreault et al., 2013; Hamdhani et al., 2020). As a result, aquatic organisms residing in effluent-receiving habitats are affected across all levels of biological organization, from molecular initiating events all the way up to population and community responses (Saaristo et al., 2014; Bahamonde et al., 2015; Fuzzen et al., 2015; McCallum et al., 2017, 2019; Du et al., 2018, 2019; McLean et al., 2019; Mehdi et al., 2018, 2021; Lau et al., 2021). As urban populations continue to grow, so too will the reliance on WWTPs, and by extension, the concerns regarding the impacts of their effluents on aquatic ecosystems (Sumpter, 2009; Bernhardt et al., 2017).

To date, most studies on the impacts of WWTP effluents have been conducted during warmer months of the year, and as a result, it is unclear whether similar impacts occur at colder temperatures, and if so, to what degree. In many parts of the world, winter is a dominant season, with its effects lasting 4–8 months of the year; therefore, understanding the impacts of such a ubiquitous contaminant as wastewater effluent in a season as dominant as winter is of crucial importance. Additionally, the effectiveness of WWTPs and therefore the quality of their effluents is poorer at colder temperatures (i.e., winter) than at warmer temperatures (i.e., summer; Vieno et al., 2005; Sui et al., 2011; Yu et al., 2013; Kot-Wasik et al., 2016). This is mainly due to poorer influent degradation at colder temperatures as well as elevated usage of PPCPs, caffeine, and health products during winter (Vieno et al., 2005; Sui et al., 2011; Yu et al., 2013; Kot-Wasik et al., 2016). The higher concentrations of PPCPs, nitrogenous waste products, and nutrients, and the overall poorer quality of wastewater effluent during winter suggests that the impacts on aquatic organisms may also be greater during winter. Further, effluents discharged during winter can increase water temperature by as much as 5–10 °C in effluent-receiving environments, potentially providing thermal refuge for aquatic organisms (Environment Canada, 2001; Kinouchi et al., 2007). Taken together, the effects of thermal pollution and nutrient enrichment may increase food availability, potentially causing wastewater outfalls to act as ecological traps, particularly during winter, when the impacts of wastewater effluent exposure may be magnified (McCallum et al., 2019; Mehdi et al., 2021).

While impacts of wastewater effluent have been well established on the individuals, comparatively, few studies have addressed how wastewater may impact aquatic populations and communities. This is surprising given how relevant such ecological endpoints are in determining habitat quality and evaluating risks, especially in environments impacted by anthropogenic disturbances (Fausch et al., 1990; Cvetkovic et al., 2010). One strategy for examining the impacts of wastewater effluent on ecosystem health is the use of aquatic benthic macroinvertebrates as bioindicators. Benthic macroinvertebrates are commonly used as bioindicators of water quality in rivers and lakes as they (i) are highly diverse and range widely in sensitivity to disturbances, (ii) typically have small home ranges, and (iii) are easily collected and identified (Krumhansl et al., 2015; Resh and Unzicker, 1975; Jones et al., 2007). Benthic macroinvertebrates also play an important role in the transformation of nutrients (Krumhansl et al., 2015; Gleason and Rooney, 2017) and are an important food source for fish, amphibians, and birds (Covich et al., 1999). Although little is known about the impacts of wastewater effluent on benthic macroinvertebrate communities, a few notable studies have demonstrated that wastewater effluent can indeed affect food web structure and function through shifts in community composition and leaf litter composition, changes in trophic status of receiving systems, and changes in biodiversity and in biological integrity indices (Ortiz et al., 2005; Englert et al., 2013; Huong et al., 2017; Burdon et al., 2019; Peschke et al., 2019; dos Reis Oliveira et al., 2020; Jesus et al., 2020). However, as mentioned above, the seasonal impacts posed by wastewater effluent on ecosystems have rarely been investigated, particularly during the winter – a season seldom studied in ecotoxicology.

The aim of the present study was therefore to compare the impact of wastewater treatment plant effluent on benthic macroinvertebrate communities between summer and winter. To do this, we examined the impacts of wastewater effluent on benthic macroinvertebrate communities near a large and small WWTP during summer and winter. The two WWTPs are both located within the Hamilton Harbour watershed in Ontario, Canada, one of 43 areas of concern under the Great Lakes Water Quality Agreement (2012). Wastewater pollution is a stressor of special concern in Hamilton Harbour, as it is estimated that ~50% of its non-lake inflow is of WWTP origin (Lawrence et al., 2004; Government of Canada, 2017). Benthic macroinvertebrate samples were collected along a distance and contamination gradient from each WWTP, thereby allowing us to assess the longitudinal effects of wastewater effluent in both summer and winter. Additionally, we measured a suite of water quality parameters and habitat quality characteristics to assess the abiotic impacts of wastewater contamination. We predicted that wastewater would significantly impair the physical and chemical quality of effluent-receiving environments, thereby affecting benthic macroinvertebrate communities at these impacted sites (Hamdhani et al., 2020; van der Meer et al., 2021). Because the continuous discharge of wastewater effluents leads to the degradation of benthic habitats in effluent-receiving environments, we expected sites closer to the effluent outfalls to have reduced richness and diversity of benthic macroinvertebrates, and be most compositionally distinct from sites farther away (Walsh et al., 2005). Since effluent quality and therefore, water quality of receiving environments would likely be worse during the winter, we expected the associated impacts on benthic macroinvertebrate communities to be more apparent during the winter.

2. Materials and methods

2.1. Sampling regime

This study was conducted during the summer of 2018 and the winter of 2019–20. Benthic macroinvertebrates were collected using artificial substrates deployed at 10 sites located along the effluent gradients of the Dundas and Woodward WWTPs discharging into Hamilton Harbour (further described in Sections 2.1.1 and 2.1.2; Fig. 1). Site selection was based on accessibility in both summer and winter and because these sites were part of a long-term research program (McCallum et al., 2019; Mehdi et al., 2021; Nikel et al., 2021).

2.1.1. Large Woodward WWTP

The Woodward WWTP is a secondary conventional activated sludge plant that serves ~480,000 people in Hamilton, Stoney Creek, and Ancaster, Canada; it has a daily capacity of 409 million litres, making it the largest WWTP in Hamilton (City of Hamilton, 2019). This plant releases its effluent into the Red Hill Creek which connects to the southeastern corner of Hamilton Harbour (Fig. 1A). Five sites were sampled along the effluent gradient of the Woodward WWTP, three of which were downstream: WDS1 (outfall), WDS2 (350 m), and WDS3 (850 m), and two of which were upstream located in Red Hill Creek: WUS1 (1400 m upstream) and WUS2 (1000 m upstream). Two reference upstream sites were selected because in 2022, one of the reference sites (WUS2) will become the new outfall site of the Woodward WWTP as part of ongoing upgrades to the plant (City of Hamilton, 2019). Therefore, our study may serve as a baseline of the conditions of benthic macroinvertebrate communities and water quality prior to the upgrades.

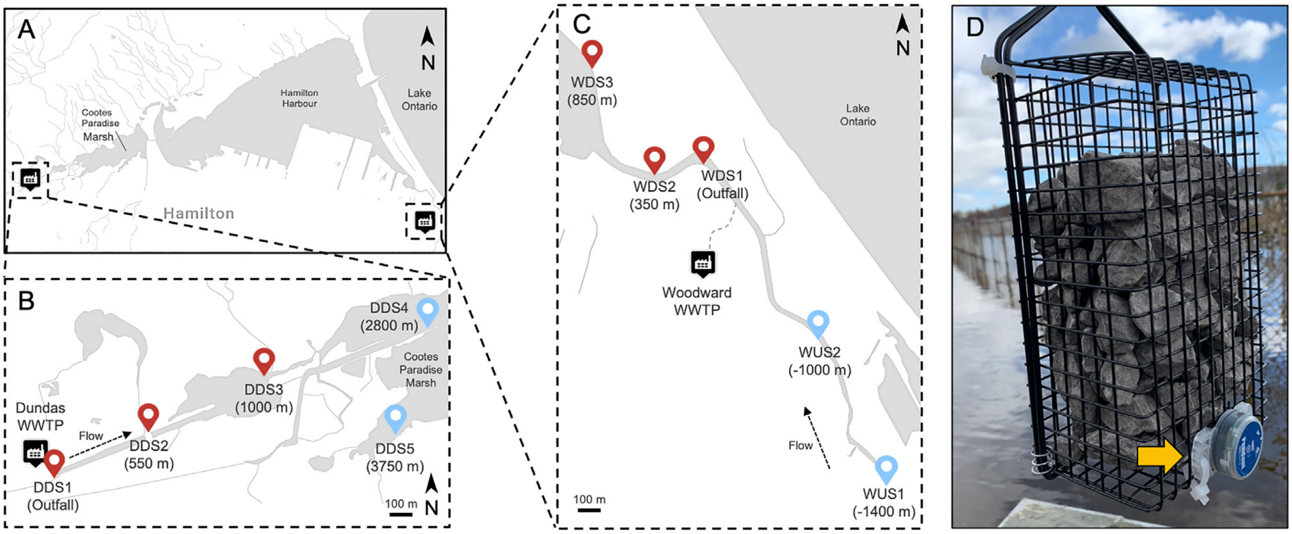


Fig. 1. Maps showing A) the location of the Dundas and Woodward WWTPs in Hamilton Harbour as well as a close-up view of the sampling sites: B) the Dundas WWTP (with an arrow indicating the direction of flow in the Desjardins Canal) and C) the Woodward WWTP (with the grey dotted line indicating the wastewater outflow from the plant and an arrow indicating the direction of flow in the Red Hill Creek). D) Photograph of a representative rock basket filled with substrate and equipped with a temperature logger (see arrow) before deployment. Sampling sites in red are in the direct flow of the effluent outfall, whereas sites in blue are not in the direct flow of the effluent outfall. Sites were named as follows: Dundas downstream (DDS); WUS (Woodward upstream); WDS (Woodward downstream).

The Woodward WWTP is situated in a heavily industrialized part of Hamilton (East Hamilton Harbour), and all sampling sites were anthropogenically modified. All sites were in close proximity to urban structures, showed clear modifications to the shoreline, had relatively narrow riparian zones, and substrate was predominantly comprised of cobble and boulder in the downstream sites and cobble and silt in the upstream reference sites. See Supplementary Material Table S1 for further details on habitat characteristics of our sampling sites.

2.1.2. Small Dundas WWTP

The smaller Dundas WWTP is a conventional activated sludge plant with tertiary filtration that serves the majority of the Dundas population (~30,000 people) and has a daily capacity of 18.2 million litres. It is located on the west end of Cootes Paradise Marsh, the largest wetland of western Lake Ontario (City of Hamilton, 2019). Effluent from the plant is discharged along an old shipping corridor, the Desjardins Canal, located on the westernmost end of Cootes Paradise Marsh (Theysmeyer and Bowman, 2017). Three of the sites sampled were in the direct flow of the effluent: DDS1 (outfall), DDS2 (550 m downstream), and DDS3 (1000 m downstream). Additionally, because the WWTP outfall is located at the head of the stream, there were no upstream sites of the Dundas WWTP, therefore two distant, but downstream reference sites were sampled: DDS4 (2800 m downstream) and DDS5 (3750 m downstream). Neither of these sites were in the direct flow of the effluent, therefore effluent exposure was less than in sites that were in the direct flow of the effluent (DDS1, DDS2, and DDS3).

The Dundas WWTP is situated in a less industrialized part of Hamilton (West Hamilton Harbour) and has a much larger surrounding riparian zone than the Woodward WWTP. Sites closest to the outfall of the Dundas WWTP (DDS1 and DDS2) were the most anthropogenically disturbed with clear modifications to the shoreline and were in close proximity to urban structures. Sites farther away from the outfall (DDS3–DDS5) were more natural, surrounded by wetland and natural forest habitats, and were less disturbed than sites near the outfall. See Supplementary Material Table S1 for further details on habitat characteristics of our sampling sites.

2.2. Benthic macroinvertebrate collection, enumeration, and identification

Benthic macroinvertebrates were sampled using wire baskets filled with 2 kg of prewashed crushed granite rocks (mean ± SE surface area of all rocks within a basket = 24.6 ± 1.21 cm^2; Fig. 1D). The prewashed and premeasured rocks in the wire baskets were used to standardize the type and amount of available substrate across the sampling sites (actual site substrate included: boulder, cobble, gravel, sand, and silt; see Supplementary Table S1 for details on substrate and habitat characteristics). Rock baskets were placed at the sampling sites by lowering them onto the substrates, at a depth of 0.5–1.0 m and within 2 m from the shoreline. Baskets were left in contact with the existing sediment and suspended by rope to prevent them from sinking into the substrate if the substrate was too soft. The baskets were left to be colonized by benthic macroinvertebrates for 8 weeks in both seasons. In the summer of 2018, 60 rock baskets (n = 6/site) were deployed from July 10th until September 4th at the Dundas WWTP sampling sites and from July 15th until September 10th at the Woodward WWTP sampling sites; of these, 51 were retrieved. Winter baskets were initially deployed in December 2018, however, high water levels led to difficulties with their retrieval causing the winter sample collection to be delayed until winter 2019–20. Forty-five rock baskets (n = 5/site) were deployed from November 16th, 2019 to January 10th, 2020 at the Dundas WWTP sampling sites and from November 17th, 2019 to January 11th, 2020 at the Woodward WWTP sampling sites; of these, 38 were retrieved. Rock baskets could not be deployed in the winter at one of the reference sites (DDS5; Dundas WWTP) due to ice cover and all rock baskets deployed at WDS3 (Woodward WWTP) in the winter were lost due to vandalism. See Supplementary Table S2 for additional details on rock basket deployment. Each rock basket was retrieved by lifting it out of the water inside a D-net (500 μm mesh size) to prevent sample loss, and then the rocks were washed into a 500 μm-sized sieve to collect the invertebrates. Samples were immediately preserved in 10% sugar-buffered formalin before being transferred into 70% ethanol. For the majority of samples, all invertebrates were identified and counted. However, if the samples were too dense (>400 individuals in the first quadrant), then they were subsampled in halves or in quarters (see Supplementary Table S2 for details on subsampling). For enumeration, samples were emptied into a large dishpan and benthic macroinvertebrates were identified to the lowest practical taxonomic level (family) following West Virginia Department of Environmental Protection (n.d.), St.

Lawrence River Institute Environmental Sciences (2005), and Witty and Sarrazin-Delay (2014).

2.3. Habitat characterization and water quality

Habitat characteristics were assessed based on the protocols of McCallum et al. (2019) and a subset of metrics of the Qualitative Habitat Evaluation Index (Taft and Koncelik, 2006; Strickland et al., 2010). At each sampling site, the following parameters were assessed: water depth, water clarity (Secchi disk), substrate type, sediment particle size, shoreline slope, degree of sinuosity, degree of anthropogenic modifications, riparian zone width, degree of estimated bank erosion, and the presence of any aquatic plants (see Supplementary Table S1).

At each site, four times in summer and three times in winter, we measured the following water quality parameters: water temperature and dissolved oxygen (YSI ProODO), pH, conductivity, total dissolved solids (TDS), and salinity (Oakton multiparameter Testr); (Table 1). Also, long-term temperature data were collected for 14 days using HOBO Pendant MX temperature loggers (Onset Computer Corp) deployed at the outfall site and a reference site of each WWTP in both seasons at a depth between 0.5 and 1.0 m (Fig. 1D). At each site, 1 L water samples were collected at mid-water depth using a 2.2 L Van Dorn sampler (Wildco Alpha) and later analyzed for total ammonia + ammonium, nitrate, nitrite, ortho-phosphate, total phosphorus, and total Kjeldahl nitrogen by the City of Hamilton Environmental Laboratory (methods as in McCallum et al., 2019). Additionally, 24-h composite samples of the effluent were collected twice a week directly from each WWTP just before discharge during the summer and winter of 2019 (summer: $n_{Dundas} = 7$, $n_{Woodward} = 8$; winter: $n_{Dundas} = 8$, $n_{Woodward} = 8$). The following water quality parameters were measured in the composite effluent samples: total suspended solids, biochemical oxygen demand, total phosphorus, total Kjeldahl nitrogen, ammonia, nitrate, nitrite, and *Escherichia coli* (measurements provided by the City of Hamilton and can be found in Supplementary Table 3).

2.4. Statistical analysis

Statistical analyses were performed using R (version 4.0.4; R Core Team, 2021) and graphics were made with Prism (version 9) and R. Water quality parameters were analyzed collectively using a permutation MANOVA with 5000 permutations to assess the effects of proximity to the outfall and season. For all biodiversity metrics calculated, we used each rock basket to represent a replicate and an individual observation. Total abundance, family richness, and family diversity (Shannon's Index) were analyzed (after being log-transformed due to heterogeneity of variance) using permutation linear models (PLMs) with 5000 iterations from the lme4 and predictmeans packages (Bates et al., 2015; Luo et al., 2020). We analyzed the response variables for each WWTP separately because the sampling site order differed between the two plants. At the Woodward WWTP, sampling site type (i.e., upstream or downstream; categorical), season (summer or winter; categorical), and their interaction were included in the model. Whereas at the Dundas WWTP, sampling site order (numeric), season (categorical), and their interaction were included in the model. This type of analysis allowed for response variables to be interpreted along a '*gradient of contamination*' at the Dundas WWTP and as '*upstream versus downstream*' at the Woodward WWTP. Principal Coordinate Analysis (PCoA) with a Bray-Curtis dissimilarity matrix were used to analyze benthic macroinvertebrate community composition differences between sites and seasons for each WWTP (Oksanen et al., 2019). PCoA biplots with 80% confidence ellipses overlaid on each site were used to visualize beta diversity differences across sites within each WWTP and season (Oksanen et al., 2019). Community composition differences across sites and between seasons were further analyzed using a permutation ANOVA with 5000 permutations using adonis2 (Vegan package; Oksanen et al., 2019). Similarity percentages analysis (SIMPER; Oksanen et al., 2019) assisted with identifying which family groups were driving the between-site differences in community composition in each season. While all benthic macroinvertebrate families sampled were included in the analysis, only those that contributed ≥5% to the total abundance were further interpreted using permutation tests (n = 5000). In all analyses, a difference was deemed significant when $p < 0.05$.

3. Results

3.1. Water quality

3.1.1. Large Woodward WWTP

Water quality differed significantly between downstream (WDS1, WDS2, and WDS3) and upstream sites (WUS1 and WUS2) in both seasons at the Woodward WWTP (Table 1). Across all sites, pH, conductivity, total dissolved solids, salinity, and total ammonia nitrogen ($p = 0.08$) were higher in the winter, whereas only water temperature was higher in the summer. At the downstream sites in summer, total nitrogen, total nitrate nitrogen, total phosphorus, and soluble reactive phosphorus were all significantly higher relative to sites upstream, while the opposite was true for dissolved oxygen and pH. Whereas at the downstream sites in winter, water temperature, total nitrogen, total ammonia nitrogen, total nitrate nitrogen, total phosphorus, and soluble reactive phosphorus were all higher relative to the upstream sites, the opposite was true for dissolved oxygen and pH (all contrasts were $p < 0.05$ unless otherwise stated). Data from the temperature loggers revealed no difference in water temperature between the outfall site (WDS1) and the reference site (WUS2) in the summer (Linear Model, $t_{Summer} = 0.42, p = 0.68$); however, during the winter, the outfall site was on average ~9 °C warmer than the upstream site (Linear Model, $t_{Winter} = 22.30, p < 0.001$).

3.1.2. Small Dundas WWTP

Similar to the Woodward WWTP, water quality varied significantly with distance from the effluent outfall in both seasons (Table 1). Across all sites, water temperature, total phosphorus, and soluble reactive phosphorus were all higher in summer, whereas pH, total nitrogen, total ammonia nitrogen, total nitrate nitrogen, conductivity, total dissolved solids, and salinity were higher in winter. In summer, total nitrogen, total nitrate nitrogen, conductivity, total dissolved solids, and salinity were all highest near the outfall site, and decreased with distance from the WWTP, while pH, temperature, and soluble reactive phosphorus increased with distance from the outfall. In winter, water temperature, total nitrogen, total ammonia nitrogen, total nitrate nitrogen, conductivity, total dissolved solids, and salinity were all highest near the outfall and decreased with distance from the treatment plant, while only pH increased with distance from the outfall. Temperature data loggers revealed that the outfall (DDS1) and reference (DDS4) sites had similar water temperatures during summer (Linear Model, $t_{Summer} = 1.49, p = 0.15$), but the outfall site was on average ~8 °C warmer than the reference site in winter (Linear Model, $t_{Winter} = 25.25, p < 0.001$).

3.2. Benthic macroinvertebrate community metrics

3.2.1. Large Woodward WWTP

At the Woodward WWTP sampling sites, we collected 36,854 benthic macroinvertebrates (mean ± SE = ~929 ± 189/basket in summer and ~681 ± 229/basket in winter; Table 2). Overall, benthic macroinvertebrate abundance and family richness did not significantly differ between summer and winter (PLM; $t_{Abundance(1,41)} = 1.04$ $p = 0.30$; $t_{Richness(1,41)} = 1.48, p = 0.09$; Fig. 2A and B; S1A and S1B). However, family diversity was significantly lower in winter than in summer (PLM; $t_{Diversity\ (1,41)} = -3.98$, $p < 0.001$; Fig. 2E and F). Benthic macroinvertebrate abundance was higher in sites downstream of the effluent outfall compared to upstream sites in both summer (PLM; $t_{(1,41)} = 7.35, p < 0.001$; ~21 times; Fig. 2A) and winter ($t_{(1,41)} = 4.64, p < 0.001$; ~149 times; Fig. 2B). In contrast, family diversity was significantly lower at sites downstream of the outfall relative to those upstream in both seasons (PLM, $t_{Summer(1,41)} = -5.57, p < 0.001$;

Table 1

Mean (± SE) of water quality parameters measured at the sampling sites of the Woodward and Dundas WWTPs in summer | winter. Water quality parameters shown are water temperature, dissolved oxygen saturation (DO), pH, conductivity, total dissolved solids (TDS), salinity, total ammonia nitrogen (TAN), total nitrate nitrogen (TNN), total nitrogen (TN), soluble reactive phosphorus (SRP), and total phosphorus (TP). Data based on measurements in the summer (n = 4/site) and winter (n = 3/site). Water quality data previously reported in Mehdi et al. (2021).

<table>
<tr><th></th><th colspan="5">Woodward WWTP</th></tr>
<tr><th></th><th>WUS1 (−1400 m)</th><th>WUS2 (−1000 m)</th><th>WDS1 (Outfall)</th><th>WDS2 (350 m)</th><th>WDS3 (850 m)</th></tr>
<tr><td>Water temperature (°C)</td><td>23.25 ± 1.28 | 4.43 ± 1.08</td><td>23.25 ± 0.47 | 4.13 ± 1.07</td><td>21.72 ± 0.37 | 11.3 ± 0.95</td><td>22.17 ± 0.24 | 10.7 ± 0.83</td><td>22.65 ± 0.42 | 9.76 ± 0.29</td></tr>
<tr><td>DO (%)</td><td>93.55 ± 12.98 | 95.63 ± 0.08</td><td>75.42 ± 24.53 | 92.86 ± 2.53</td><td>74.2 ± 2.6 | 69.03 ± 1.26</td><td>69.35 ± 4.47 | 68.9 ± 0.66</td><td>63.32 ± 4.1 | 67.26 ± 2.87</td></tr>
<tr><td>pH</td><td>8.13 ± 0.18 | 8.7 ± 0.31</td><td>8.05 ± 0.23 | 8.65 ± 0.42</td><td>7.07 ± 0.1 | 7.77 ± 0.23</td><td>7.08 ± 0.12 | 7.4 ± 0.09</td><td>7.05 ± 0.1 | 7.55 ± 0.09</td></tr>
<tr><td>Conductivity (μS)</td><td>1113 ± 220.58 | 2021.66 ± 349.31</td><td>1147.25 ± 207.64 | 2351.33 ± 288.89</td><td>1166.25 ± 43.47 | 2021.33 ± 360.99</td><td>1148 ± 53.24 | 2033.66 ± 298.55</td><td>1147.25 ± 56.91 | 1824 ± 288.92</td></tr>
<tr><td>TDS (ppm)</td><td>779.75 ± 156.15 | 1606.66 ± 273.57</td><td>812 ± 147.65 | 1670 ± 205.5</td><td>828 ± 30.88 | 1463.33 ± 238.35</td><td>816.75 ± 36.83 | 1443.33 ± 218.8</td><td>815 ± 40.7 | 1100.66 ± 90.11</td></tr>
<tr><td>Salinity (ppm)</td><td>550.25 ± 99.67 | 1110 ± 180.36</td><td>534.75 ± 100.49 | 1153.33 ± 142.4</td><td>540.25 ± 21.01 | 1011.33 ± 174.49</td><td>534 ± 25.2 | 1001.66 ± 159.17</td><td>534 ± 28.08 | 947.66 ± 121.54</td></tr>
<tr><td>TAN (mg/L)</td><td>0.04 ± 0.01 | 0.02 ± 0.01</td><td>0.12 ± 0.03 | 0.04 ± 0.01</td><td>0.46 ± 0.18 | 2.32 ± 1.65</td><td>0.89 ± 0.43 | 2.23 ± 1.53</td><td>1.2 ± 0.48 | 2.26 ± 1.42</td></tr>
<tr><td>TNN (mg/L)</td><td>1.66 ± 0.66 | 1.15 ± 0.06</td><td>2.09 ± 0.8 | 1.09 ± 0.13</td><td>11.25 ± 0.73 | 10.88 ± 0.5</td><td>9.54 ± 1.16 | 10.36 ± 0.71</td><td>9.14 ± 1.04 | 9.52 ± 1.16</td></tr>
<tr><td>TN (mg/L)</td><td>2.62 ± 0.95 | 1.54 ± 0.09</td><td>2.81 ± 0.86 | 1.5 ± 0.09</td><td>13.14 ± 1.03 | 16.14 ± 3.21</td><td>12.18 ± 1.41 | 15.13 ± 3.66</td><td>12.19 ± 1.76 | 14.62 ± 4.01</td></tr>
<tr><td>SRP (mg/L)</td><td>0 ± 0 | 0.02 ± 0.02</td><td>0.02 ± 0.01 | 0 ± 0</td><td>0.22 ± 0.04 | 0.18 ± 0.05</td><td>0.22 ± 0.05 | 0.18 ± 0.05</td><td>0.22 ± 0.05 | 0.19 ± 0.05</td></tr>
<tr><td>TP (mg/L)</td><td>0.15 ± 0.06 | 0.08 ± 0.02</td><td>0.13 ± 0.02 | 0.08 ± 0.01</td><td>0.45 ± 0.04 | 0.44 ± 0.03</td><td>0.4 ± 0.05 | 0.37 ± 0.05</td><td>0.36 ± 0.06 | 0.36 ± 0.06</td></tr>
<tr><th></th><th colspan="5">Dundas WWTP</th></tr>
<tr><th></th><th>DDS1 (Outfall)</th><th>DDS2 (550 m)</th><th>DDS3 (1000 m)</th><th>DDS4 (2800 m)</th><th>DDS5 (3750 m)</th></tr>
<tr><td>Water temperature (°C)</td><td>21.05 ± 1.35 | 9.96 ± 1</td><td>23.32 ± 1.15 | 7.66 ± 1.19</td><td>24.22 ± 1.4 | 4.86 ± 2.15</td><td>23.65 ± 2.24 | 3.23 ± 1.15</td><td>26.7 ± 2.43 | 3.6 ± 2.7</td></tr>
<tr><td>DO (%)</td><td>124.02 ± 13.46 | 91.06 ± 8.65</td><td>134.32 ± 23.9 | 98.23 ± 9.6</td><td>90.7 ± 34.57 | 109.86 ± 2.81</td><td>86.97 ± 15.28 | 100.83 ± 2.67</td><td>132.42 ± 48.53 | 107.93 ± 4.17</td></tr>
<tr><td>pH</td><td>7.57 ± 0.22 | 7.69 ± 0.38</td><td>7.88 ± 0.54 | 7.84 ± 0.33</td><td>7.49 ± 0.45 | 8.4 ± 0.41</td><td>8.21 ± 0.29 | 8.78 ± 0.36</td><td>8.33 ± 0.54 | 8.8 ± 0.28</td></tr>
<tr><td>Conductivity (μS)</td><td>1188.25 ± 91.89 | 1452 ± 130.03</td><td>1149.25 ± 93.2 | 1530 ± 159.48</td><td>1134.25 ± 149.38 | 1495.33 ± 142.33</td><td>943.5 ± 201.07 | 942 ± 157.08</td><td>885.75 ± 129.63 | 966.66 ± 169.78</td></tr>
<tr><td>TDS (ppm)</td><td>844.5 ± 68.14 | 1005.33 ± 82.12</td><td>818.75 ± 63.94 | 1086.33 ± 112.07</td><td>806.75 ± 105.7 | 1065.33 ± 101.71</td><td>670.25 ± 141.5 | 670.33 ± 113.03</td><td>626.25 ± 91.45 | 690 ± 121.5</td></tr>
<tr><td>Salinity (ppm)</td><td>562.5 ± 58.59 | 718.66 ± 59.87</td><td>544.25 ± 41.39 | 756 ± 65.93</td><td>536.75 ± 61.68 | 732.33 ± 49.66</td><td>441 ± 89.22 | 461.66 ± 102.75</td><td>414 ± 54.06 | 476.33 ± 104.64</td></tr>
<tr><td>TAN (mg/L)</td><td>0.03 ± 0.01 | 0.5 ± 0.75</td><td>0.05 ± 0.02 | 0.22 ± 0.27</td><td>0.06 ± 0.04 | 0.13 ± 0.1</td><td>0.06 ± 0.07 | 0.04 ± 0.02</td><td>0 ± 0 | 0.02 ± 0.01</td></tr>
<tr><td>TNN (mg/L)</td><td>15.35 ± 1.53 | 17.09 ± 2.03</td><td>11.26 ± 3.64 | 16.1 ± 1.76</td><td>4.84 ± 3.14 | 11.62 ± 2.24</td><td>0.49 ± 0.62 | 1.06 ± 0.33</td><td>0 ± 0 | 0.69 ± 0.35</td></tr>
<tr><td>TN (mg/L)</td><td>16.25 ± 1.03 | 18.53 ± 1.2</td><td>12.49 ± 3.36 | 17.03 ± 1.77</td><td>6.43 ± 3.04 | 13.12 ± 1.68</td><td>1.52 ± 0.79 | 1.64 ± 0.39</td><td>1.38 ± 0.44 | 1.21 ± 0.43</td></tr>
<tr><td>SRP (mg/L)</td><td>0 ± 0 | 0 ± 0</td><td>0 ± 0 | 0 ± 0</td><td>0.01 ± 0.02 | 0 ± 0</td><td>0.03 ± 0.03 | 0 ± 0</td><td>0.03 ± 0.04 | 0 ± 0</td></tr>
<tr><td>TP (mg/L)</td><td>0.11 ± 0.04 | 0.07 ± 0.01</td><td>0.11 ± 0.04 | 0.07 ± 0.02</td><td>0.18 ± 0.06 | 0.07 ± 0</td><td>0.11 ± 0.01 | 0.06 ± 0.03</td><td>0.19 ± 0.1 | 0.05 ± 0</td></tr>
</table>

Table 2
Benthic macroinvertebrate counts at each sampling site (abundances are denoted as Summer | Winter, "-" indicates no information for that specific site). Count data are shown as the total number of invertebrates collected in all rock baskets per site (see Supplementary Table S3 for sample sizes by site and season).

<table>
<tr><th rowspan="2">Organism type</th><th rowspan="2">Order</th><th rowspan="2">Family</th><th colspan="5">Woodward WWTP</th><th colspan="5">Dundas WWTP</th></tr>
<tr><th>WUS1 (−1400 m)</th><th>WUS2 (−1000 m)</th><th>WDS1 (outfall)</th><th>WDS2 (350 m)</th><th>WDS3 (850 m)</th><th>DDS1 (outfall)</th><th>DDS2 (550 m)</th><th>DDS3 (1000 m)</th><th>DDS4 (2800 m)</th><th>DDS5 (3750 m)</th></tr>
<tr><td>Amphipods</td><td>Amphipoda</td><td>Gammaridae</td><td>27 | 66</td><td>23 | 1032</td><td>0 | 0</td><td>6 | 2</td><td>0 | –</td><td>2 | 6</td><td>57 | 221</td><td>56 | 216</td><td>9344 | 1046</td><td>160 | –</td></tr>
<tr><td>Isopods</td><td>Isopoda</td><td>Asellidae</td><td>42 | 33</td><td>41 | 47</td><td>7711 | 10,966</td><td>5260 | 912</td><td>4164 | –</td><td>95 | 16</td><td>98 | 70</td><td>719 | 9</td><td>100 | 58</td><td>479 | –</td></tr>
<tr><td>Leeches</td><td>Arhynchobdellida</td><td>Erpobdellidae</td><td>63 | 6</td><td>32 | 6</td><td>56 | 45</td><td>527 | 108</td><td>100 | –</td><td>0 | 2</td><td>31 | 0</td><td>26 | 0</td><td>4 | 1</td><td>112 | –</td></tr>
<tr><td></td><td>Rhynchobdellida</td><td>Glossiphoniidae</td><td>20 | 0</td><td>8 | 1</td><td>0 | 0</td><td>483 | 0</td><td>136 | –</td><td>0 | 0</td><td>1 | 0</td><td>6 | 0</td><td>0 | 0</td><td>45 | –</td></tr>
<tr><td>Seg. Worms</td><td>Haplotaxida</td><td>Naididae</td><td>115 | 14</td><td>8 | 16</td><td>10 | 6</td><td>11| 4</td><td>0 | –</td><td>8 | 2</td><td>1 | 3</td><td>15 | 0</td><td>0 | 0</td><td>149 | –</td></tr>
<tr><td>Flatworms</td><td>Tricladida</td><td>Planariidae</td><td>0 | 0</td><td>1 | 2</td><td>1 | 0</td><td>46 | 4</td><td>4 | –</td><td>0 | 2</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td>Snails</td><td>Basommatophora</td><td>Physidae</td><td>14 | 1</td><td>15 | 3</td><td>29 | 66</td><td>118 | 5</td><td>68 | –</td><td>8 | 1</td><td>5 | 0</td><td>2 | 4</td><td>4 | 14</td><td>9 | –</td></tr>
<tr><td></td><td></td><td>Planorbidae</td><td>1 | 0</td><td>3 | 0</td><td>0 | 0</td><td>10 | 0</td><td>72 | –</td><td>1 | 0</td><td>0 | 0</td><td>1 | 0</td><td>4 | 0</td><td>4 | –</td></tr>
<tr><td></td><td></td><td>Lymnaeidae</td><td>1 | 0</td><td>1 | 1</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 |0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td>Heterostropha</td><td>Valvatidae</td><td>1 | 0</td><td>1 | 1</td><td>0 | 0</td><td>0 | 0</td><td>2348 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>5 | –</td></tr>
<tr><td></td><td>NA</td><td>Snails w/ No Shell</td><td>0 | 0</td><td>1 | 0</td><td>4 | 16</td><td>11 | 2</td><td>0 | –</td><td>0 | 0</td><td>0 | 1</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td>Neotaenioglossa</td><td>Hydrobiidae</td><td>28 | 4</td><td>107 | 4</td><td>0 | 0</td><td>125 | 13</td><td>684 | –</td><td>0 | 0</td><td>17 | 0</td><td>9 | 0</td><td>52 | 0</td><td>52 | –</td></tr>
<tr><td></td><td>Venerida</td><td>Sphaeriidae</td><td>46 | 13</td><td>12 | 61</td><td>0 | 0</td><td>0 | 0</td><td>4 | –</td><td>0 | 0</td><td>0 | 1</td><td>1 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td>Beetles</td><td>Coleoptera</td><td>Dryopidae</td><td>0 | 0</td><td>1 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>10 | 0</td><td>0 | 0</td><td>40 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Haliplidae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>2 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Hydrophilidae</td><td>5 | 0</td><td>42 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>2 | 1</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Elmidae</td><td>1 | 0</td><td>0 | 0</td><td>0 | 0</td><td>3 | 0</td><td>0 | –</td><td>0 | 0</td><td>4 | 3</td><td>1 | 1</td><td>32 | 1</td><td>2 | –</td></tr>
<tr><td></td><td></td><td>Dytiscidae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 1</td><td>7 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td>True Bugs</td><td>Hemiptera</td><td>Gerridae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>1 | 0</td><td>5 | 0</td><td>8 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Veliidae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>1 | 0</td><td>14 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Corixidae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>8 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Belostomatidae</td><td>0 | 0</td><td>0 | 1</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 4</td><td>0 | 1</td><td>4 | 0</td><td>0 | –</td></tr>
<tr><td>Flies</td><td>Diptera</td><td>Chironomidae</td><td>8 | 16</td><td>0 | 104</td><td>334 | 15</td><td>13 | 5</td><td>0 | –</td><td>42 | 7</td><td>144 | 14</td><td>36 | 10</td><td>68 | 17</td><td>1 | –</td></tr>
<tr><td></td><td></td><td>Ceratopogonidae</td><td>0 | 1</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>24 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Tabanidae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>3 | –</td></tr>
<tr><td></td><td>Ephemeroptera</td><td>Caenidae</td><td>0 | 0</td><td>0 | 0</td><td>1 | 0</td><td>0 | 0</td><td>0 | –</td><td>5 | 2</td><td>110 | 79</td><td>3 | 0</td><td>76 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Ameletidae</td><td>0 | 0</td><td>0 | 0</td><td>5 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>1 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td>Odonata</td><td>Libellulidae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>2 | 0</td><td>6 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Coenagrionidae</td><td>90 | 2</td><td>88 | 16</td><td>20 | 2</td><td>4 | 3</td><td>0 | –</td><td>139 | 6</td><td>87 | 91</td><td>75 | 160</td><td>236 | 12</td><td>2 | –</td></tr>
<tr><td></td><td></td><td>Calopterygidae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>1 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Corduliidae</td><td>2 | 2</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>1 | 2</td><td>33 | 0</td><td>41 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Aeshnidae</td><td>0 | 0</td><td>0 | 1</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>4 | 0</td><td>0 | –</td></tr>
<tr><td></td><td>Plecoptera</td><td>Nemouridae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 1</td><td>0 | –</td></tr>
<tr><td></td><td>Trichoptera</td><td>Psychomyiidae</td><td>0 | 0</td><td>0 | 0</td><td>4 | 0</td><td>2 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Hydropsychidae</td><td>2 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Phryganeidae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 13</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Odontoceridae</td><td>0 | 1</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 3</td><td>0 | –</td></tr>
<tr><td></td><td>Megaloptera</td><td>Sialidae</td><td>4 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
<tr><td></td><td></td><td>Corydalidae</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 1</td><td>0 | 0</td><td>12 | 3</td><td>0 | –</td></tr>
<tr><td></td><td>Lepidoptera</td><td>Pyralidae</td><td>0 | 1</td><td>0 | 0</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td><td>0 | 0</td><td>0 | 1</td><td>0 | 0</td><td>0 | 0</td><td>0 | –</td></tr>
</table>

Fig. 2E; $t_{Winter(1,41)} = -5.26, p < 0.001$; Fig. 2F). Family richness on the other hand did not differ between downstream and upstream sites in either season (PLM, $t_{Summer(1,41)} = 1.50, p = 0.12$; Fig. S1A; $t_{Winter(1,41)} = 0.63, p = 0.23$; Fig. S1B).

3.2.2. Small Dundas WWTP

At the Dundas WWTP sampling sites, we collected a total of 15,082 benthic macroinvertebrates (mean ± SE = ~499 ± 149/basket in the summer and ~117 ± 23/basket in the winter; Table 2). The abundance of macroinvertebrates was significantly higher in summer than in winter (PLM; $t_{(1,40)} = 2.55, p = 0.02$; Fig. 2C and D). Similarly, family richness was higher in summer than in winter (PLM; $t_{(1,40)} = 1.42, p = 0.02$; Supplementary Fig. S1C and D), whereas family diversity did not differ between seasons (PLM; $t_{(1,40)} = 0.83, p = 0.13$; Fig. 2G and H). In contrast to the large Woodward WWTP, total abundance increased with distance from the outfall during both summer (PLM; $t_{(1,40)} = 4.92, p < 0.001$; Fig. 2C) and winter ($t_{(1,40)} = 5.47, p < 0.001$; Fig. 2D). Family diversity, however, decreased with distance from the outfall in both seasons (PLM; $t_{(Summer\ 1,40)} = -1.97, p = 0.04$; $t_{(Winter\ 1,40)} = -3.26, p < 0.001$; Fig. 2G and H). Conversely, family richness was not influenced by proximity to the outfall in either season (PLM; $t_{(Summer\ 1,40)} = 1.45, p = 0.24$; $t_{(Winter\ 1,40)} = 1.49, p = 0.14$; Supplementary Fig. S1C and D).

3.3. Benthic macroinvertebrate community composition

3.3.1. Large Woodward WWTP

During summer, we identified 24 different families of benthic macroinvertebrates in all sampling sites, and the samples were largely comprised of isopods, snails, and leeches. During winter, we identified only 20 families, and the samples were mostly comprised of isopods and amphipods. See Fig. 3A and B and Table 2 for the community makeup of benthic macroinvertebrates at each sampling site in each season. Benthic macroinvertebrate community composition differed significantly between seasons (Permutation ANOVA; $F_{(1,44)} = 7.62, p < 0.001$; Fig. 4A and B). In summer, communities downstream of the outfall were the most distinct from those upstream (PCoA; Permutation ANOVA; $F_{Summer(4,24)} = 7.35, p < 0.001$; Fig. 4A). In the winter, upstream communities displayed a degree of overlap with each other but were distinct from those at sites downstream; although the two downstream sites were distinct from one another (PCoA; Permutation ANOVA; $F_{Winter(3,19)} = 16.7, p < 0.001$; Fig. 4B). Similarity analysis indicated that differences between WDS1 (outfall) and all other sites were mainly driven by a high abundance of *Asellidae* (freshwater isopods) at WDS1 in both seasons (Table 3 and Supplementary Table S4). Furthermore, dissimilarity scores were highest between the outfall site and the two reference sites upstream, and this was observed in both summer and winter (Table 3).

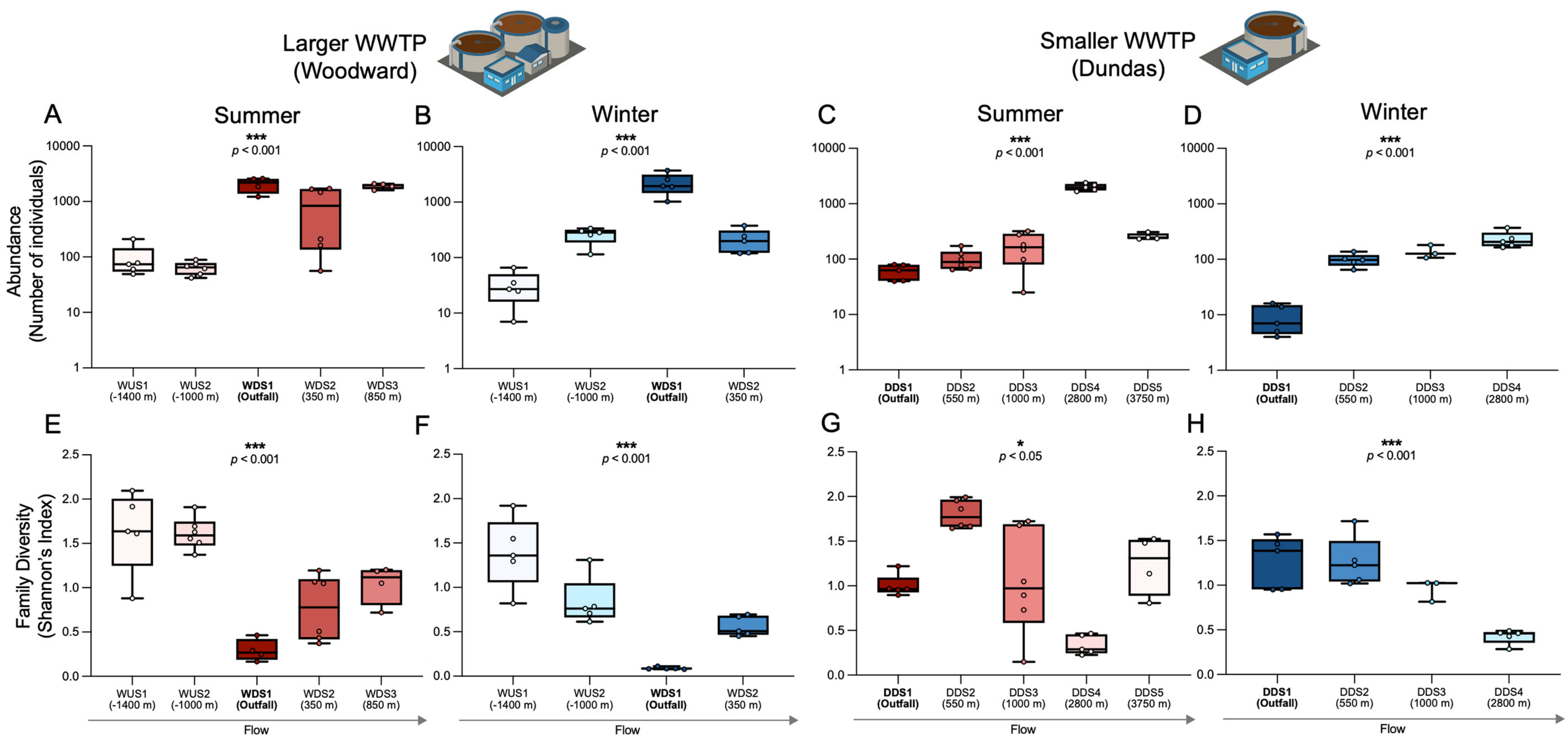


Fig. 2. Benthic macroinvertebrate abundance (on a log scale) for the Woodward WWTP in the summer (A) and winter (B), and for the Dundas WWTP in summer (C) and winter (D). Family diversity (Shannon's Index) shown for the Woodward WWTP in summer (E) and winter (F), and for the Dundas WWTP in summer (G) and winter (H). Boxplots show the median and inter-quartile range, whiskers show minimum and maximum values, and individual data points are jittered to improve visualization. In all figures, summer data are represented in red and winter data are represented in blue; the intensity of the colours signify proximity to the outfall (darker colours being closest and light colours being most distant). Direction of flow is shown by arrows under the x-axes (with the outfall site indicated in bold font). All data are presented as per basket (N = 3–6 samples/site; see Supplementary Table S3 for sample sizes by site and season). Significant effects of proximity to the outfall in Dundas and differences between upstream and downstream sites at Woodward are indicated by $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, or no significance (n.s.).

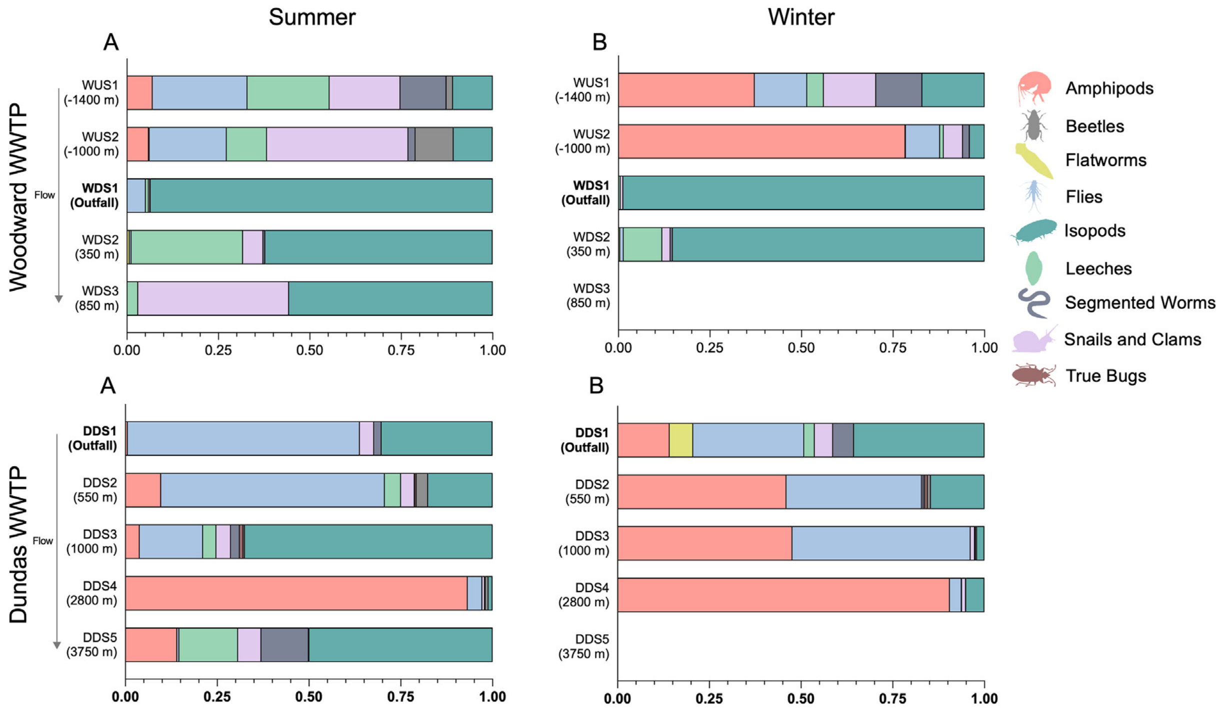


Fig. 3. Proportions of different types of benthic macroinvertebrates from the Woodward WWTP sampling sites in summer (A) and winter (B), and from the Dundas WWTP sampling sites in summer (C) and winter (D). Proportions based on total abundance of macroinvertebrates within each site and season (all basket replicates combined per site). Direction of flow is shown by arrows next to the y-axes (with the outfall site shown in bold font).

3.3.2. Small Dundas WWTP

During summer, we identified 30 families of benthic macroinvertebrates across all sampling sites, and the samples were mostly comprised of isopods, amphipods, and dipterans. In winter, we identified 21 families and like in summer, the samples were mainly comprised of isopods, amphipods, and dipterans. The community makeup of benthic macroinvertebrates at each sampling site in both seasons can be found in Fig. 3C and D and Table 2. Like the Woodward WWTP, benthic macroinvertebrate community composition at the Dundas WWTP differed significantly between seasons (Permutation ANOVA; $F_{(1,43)} = 13.7, p < 0.001$; Fig. 4C and D). In summer, the communities closest to the outfall (DDS1 and DDS2) were the most distinct from those farther away (DDS4 and DDS5), while DDS3 was an intermediate between those sites (PCoA; Permutation ANOVA; $F_{\text{Summer}(4,25)} = 10.5, p < 0.001$; Fig. 4C). During winter, the benthic macroinvertebrate community at the outfall site (DDS1) was most different from the site farthest away (DDS4); while sites in between (DDS2 and DDS3) shared a considerable amount of overlap (PCoA; Permutation ANOVA; $F_{\text{Winter}(3,17)} = 9.76, p < 0.001$; Fig. 4D). Similarity analysis further indicated which families were driving the majority of community compositional differences across sites. In summer, community differences between DDS1 and DDS2 were largely driven by the higher abundance of *Chironomidae* (midges) and *Caenidae* (square gill mayflies) at DDS2, and a higher abundance of *Coenagrionidae* (damselflies) at DDS1. Differences between DDS1 and DDS3 were driven by the higher abundance of *Coenagrionidae* at DDS1, but also by the higher abundance of *Asellidae* (isopods) at DDS3. The difference between DDS1 and DDS4 was mostly driven by *Gammaridae* (amphipods) which were considerably more abundant at DDS4. Differences between DDS1 and DDS5 were driven by *Naididae* (clitellate oligochaete worms) and *Erpobdellidae* (proboscisless leeches) being more abundant at DDS1 and *Asellidae* being more abundant at DDS5. In winter, higher abundances of both *Caenidae* and *Asellidae* were found at DDS2 compared to DDS1. Between DDS1 and DDS3, the differences were driven by a higher abundance of *Coenagrionidae* at DDS3. Differences between DDS1 and DDS4 were attributed mostly to the higher abundance of *Gammaridae* at DDS4 (Table 3 and Supplementary Table S5). Furthermore, dissimilarity scores were highest between the outfall site and the reference site(s) in both summer and winter (Table 3).

4. Discussion

In this study, we examined the impacts of wastewater effluent in summer and winter on benthic macroinvertebrate communities near two WWTPs that discharge their effluents into very different habitats. We demonstrated that the effluents from the two WWTPs impacted the biodiversity of benthic macroinvertebrates differently. In both seasons, the larger Woodward WWTP, with its less effective secondary treatment and location in a highly industrialized area, had higher abundance but lower diversity of benthic macroinvertebrates at sites downstream of the outfall compared to sites upstream. In contrast, the smaller Dundas WWTP, with its enhanced tertiary treatment and location in a wetland, had lower abundance but higher diversity of benthic macroinvertebrates near the outfall sites compared to sites farther away in both seasons. Additionally, in both seasons and at both WWTPs, community composition of benthic macroinvertebrates differed significantly between sites closer to the outfall and sites farther away. Finally, we detected significant water quality deterioration in sites closer to the outfall, with water quality generally being poorer during the winter. The degraded water quality at the impacted sites manifested in higher nutrient concentrations, conductivity, salinity, and total dissolved solids in both seasons, as well as WWTP-induced thermal pollution, whereby sites closer to the outfall were 8–9 °C warmer during winter. Although the water quality varied among seasons, we did not detect concurrent seasonal differences in benthic macroinvertebrate community responses to wastewater at either plant.

4.1. Contrasting patterns between the two WWTPs

Poor water quality and high nutrient concentrations have been associated with higher abundance but lower measures of diversity of aquatic communities (Birge et al., 1989; Hickey and Clements, 1998; Walsh et al., 2005; Brown et al., 2011; Grantham et al., 2012; Tetreault et al., 2013;

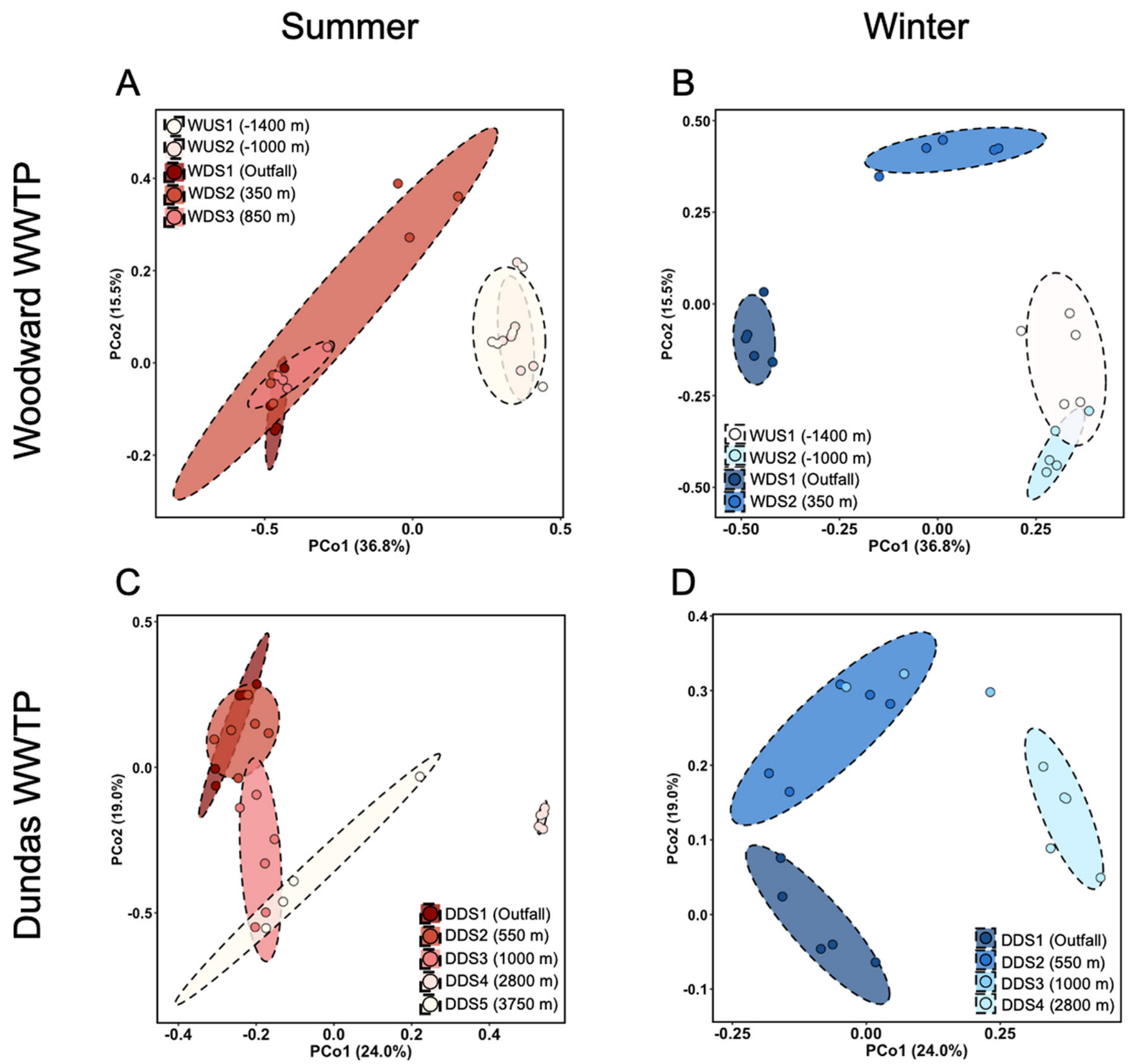


Fig. 4. Principle coordinate analysis (PCoA) ordination biplots for benthic macroinvertebrate community composition at the Woodward WWTP sites in summer (A) and winter (B), and at the Dundas WWTP sites in summer (C) and winter (D). Each site is overlaid with 80% confidence ellipses (except for Dundas DDS3 in winter due to small sample size; n < 4).

Zokan and Drake, 2015; Tuncay, 2016; McCallum et al., 2019; Jesus et al., 2020; Mehdi et al., 2021). Our results from sites along the Woodward WWTP effluent gradient are consistent with these previously reported findings. The high nutrient inputs at the Woodward WWTP appear to be supporting more benthic macroinvertebrates, and specifically, those with high tolerance to poor water quality (e.g., *Asellidae*). Interestingly, our results from along the effluent gradient of the Dundas WWTP revealed an opposite trend to that observed at the Woodward WWTP, with abundance increasing and diversity decreasing with distance from the effluent outfall. The Dundas WWTP is a much smaller plant with a higher level of treatment compared to the Woodward WWTP. Additionally, its daily effluent discharge is significantly lower than that of Woodward's (18.2 versus 409 million litres per day). Those two factors could explain why the water quality downstream of the Woodward WWTP was worse than water quality downstream of the Dundas WWTP. For example, total phosphorus, one of the main water quality indicators of productivity (Schindler, 1978; McQueen et al., 1986; Chapra and Robertson, 1977), was significantly higher in the sites downstream of the Woodward WWTP relative to sites downstream of the Dundas WWTP in both seasons, but this difference was more obvious in the winter. Furthermore, the Dundas WWTP releases its effluent into Cootes Paradise Marsh, the largest wetland west of Lake Ontario and a nature sanctuary that is of vital importance for migratory waterfowl and provides a significant habitat for many reptiles, amphibians, and fish (Leslie and Timmins, 1992; Smith and Chow-Fraser, 2010). Moreover, wetlands are known to buffer the effects of aquatic pollution as many wetland plants are able to absorb nutrients and toxic substances (Brix, 1994; Gopal, 1999; Hamoda et al., 2004). The Woodward WWTP discharges its effluent in an engineered channel, with relatively high flow rates and harder substrate (comprised mostly of boulder and cobble), and is surrounded by a heavily industrialized part of Hamilton with relatively little to no riparian zones. Taken together, the smaller size and smaller effluent footprint of the Dundas WWTP, its superior treatment, and the wetland environment receiving its effluent could help explain the contrasting biodiversity patterns observed between the two plants.

4.2. Limited differences between summer and winter patterns

Although water quality was strongly influenced by seasonality, with water quality in winter being significantly more impaired than in summer, the impacts of wastewater on benthic macroinvertebrate communities were similar between seasons. Deterioration of water quality during winter is consistent with prior studies demonstrating reduced contaminant

Table 3
Similarity percentages analysis (SIMPER) indicating the relative contribution of family groups to the overall dissimilarity score of the Woodward outfall site (WDS1) to all other Woodward sites and the Dundas outfall site (DDS1) to all other Dundas sites. Average A and B are based on the abundance for each family group at the sites being compared. Sites are ordered according to proximity to outfall and contamination load. Only families that contributed ≥5% to overall abundance are shown. Bolded values indicate significant differences between sites ($p < 0.05$).

	Summer					Winter				
Comparison	Dissimilarity	Family	Average A	Average B	Contribution	Dissimilarity	Family	Average A	Average B	Contribution
Woodward WWTP										
A. WDS1 (outfall)	54.00%	*Asellidae*	1927.75	876.67	43.27	82.07%	***Asellidae***	**2193.20**	**182.40**	**80.48**
B. WDS2 (350 m)										
A. WDS1 (outfall)	46.23%	*Asellidae*	1927.75	1041.00	22.09	NA				
B. WDS3 (850 m)		*Valvatidae*	0.00	587.00	15.14					
A. WDS1 (outfall)	98.23%	***Asellidae***	**1927.75**	**6.83**	**90.11**	98.67%	***Asellidae***	**2193.20**	**9.40**	**86.56**
B. WUS2 (−1000 m)							*Gammaridae*	0.00	206.40	9.42
A. WDS1 (outfall)	97.33%	***Asellidae***	**1927.75**	**8.40**	**88.74**	98.97%	***Asellidae***	**2193.20**	**6.60**	**96.65**
B. WUS2 (−1400 m)										
Dundas WWTP										
A. DDS1 (outfall)	61.69%	***Chironomidae***	**8.40**	**24.00**	**12.82**	89.02%	*Gammaridae*	1.20	44.20	40.81
B. DDS2 (550 m)		*Asellidae*	19.00	16.33	10.66		*Coenagrionidae*	1.20	18.20	16.43
		Coenagrionidae	**27.80**	**14.50**	**10.40**		***Caenidae***	**0.40**	**15.80**	**13.43**
		Caenidae	**1.00**	**18.33**	**9.77**		***Asellidae***	**3.20**	**14.00**	**11.45**
		Gammaridae	0.40	9.50	5.79					
A. DDS1 (outfall)	74.62%	***Asellidae***	**19.00**	**119.83**	**40.20**	92.99%	*Gammaridae*	1.20	72.00	44.02
B. DDS3 (1000 m)		***Coenagrionidae***	**27.80**	**12.50**	**13.18**		***Coenagrionidae***	**1.20**	**53.33**	**39.28**
A. DDS1 (outfall)	96.32%	***Gammaridae***	**0.40**	**1868.80**	**90.43**	94.53%	***Gammaridae***	**1.20**	**209.20**	**86.32**
B. DDS4 (2800 m)										
A. DDS1 (outfall)	86.46%	***Asellidae***	**19.00**	**119.75**	**34.80**	NA				
B. DDS5 (3750 m)		*Gammaridae*	0.40	40.00	11.51					
		Naididae	**1.60**	**37.25**	**10.34**					
		Erpobdellidae	**0.00**	**28.00**	**9.12**					
		Coenagrionidae	27.80	0.50	8.70					

removal/degradation efficiency by WWTPs at colder temperatures and increased usage and therefore concentrations of pharmaceuticals, caffeine, and other products during winter (Vieno et al., 2005; Gardarsdottir et al., 2010; ter Laak et al., 2010; Sui et al., 2011; Yu et al., 2013). The limited seasonal shifts in benthic macroinvertebrate community responses to wastewater is intriguing. During winter, many aquatic invertebrates are inactive, are only present as immobile eggs, migrate to more suitable habitats, and/or burrow into the sediment to withstand the cold/ice cover (Frouz et al., 2003; Hill et al., 2016). Additionally, some adult life stages of aquatic invertebrates, such as trichoptera and coleoptera, are known to take winter refuge in adjacent terrestrial habitats, and both of these taxa were absent in our winter samples (Chadd, 2010; Hill et al., 2016). These explanations should have led to several taxa being missed in our aquatic artificial substrate colonization technique during winter, resulting in large differences between seasons. This was evidently the case for total abundance and richness at Dundas WWTP sampling sites and for diversity at the Woodward WWTP sampling sites, as all were lower during winter. However, we did not find a strong seasonal influence on the impacts of wastewater on benthic macroinvertebrate communities; impacted sampling sites remained relatively high in abundance at Woodward and low in abundance at Dundas in both seasons.

The contrasting patterns in total macroinvertebrate abundance between the two WWTPs types could be due to bottom-up effects. The effluent released from the Woodward WWTP was of poorer quality than effluent released from the Dundas WWTP, particularly during winter. At the larger Woodward WWTP, the higher levels of nutrients, organic matter (TDS and TSS as measured in the effluent), and temperature at sites near the outfall during the winter may have allowed certain taxa to remain high in abundance via bottom-up control despite the expected winter dormancy effects described above. This was further supported by the higher fold-difference in benthic macroinvertebrate abundance between downstream and upstream sites at the Woodward WWTP in winter (~149×) compared to summer (~21×). In contrast, at the smaller Dundas WWTP, the lower abundance of benthic macroinvertebrates during winter may be attributed to the overall lower levels of total phosphorus that characterized all sampling sites. As mentioned before, phosphorus is one of the main water quality indicators of productivity (Schindler, 1978; McQueen et al., 1986; Chapra and Robertson, 1977), therefore, the lower concentrations of phosphorus measured at the Dundas sampling sites could explain the overall lower abundance of benthic macroinvertebrates in winter. However, this was not the case at Woodward, where total phosphorus levels remained relatively stable between seasons, particularly at the downstream sites. Overall, the lack of strong seasonal effects on benthic macroinvertebrate community responses to wastewater contradicted our initial predictions. These predictions were based on our previous work that demonstrated strong seasonal effects, where fish (Mehdi et al., 2021) and zooplankton (Mehdi et al., in prep) abundance were significantly higher near wastewater outfalls, but only during the winter. The different seasonal responses to wastewater effluent demonstrated by different trophic levels remains largely unexplored and warrants further research.

4.3. Conclusions

Our study is unique because we compared the impacts of wastewater effluent contamination on benthic macroinvertebrate communities and water quality between summer and winter. Despite finding major seasonal differences in water quality in effluent-receiving environments as well as finding general differences in community metrics between seasons, the effects of wastewater on benthic macroinvertebrate communities were similar in summer and winter. Interestingly, the two WWTPs sampled in our study demonstrated opposite trends in their impacts on benthic macroinvertebrate communities. Further research should investigate why and how WWTPs with different treatment capabilities and effluent-receiving environments might impact benthic communities differently. Differences in WWTPs' effluent footprints, treatment capabilities, source populations, and receiving habitats must be taken into consideration when evaluating their impacts on aquatic environments. Such research would improve the direction and precision of remediation strategies in restoring aquatic communities in effluent-receiving habitats. Additionally, studies of this kind demonstrate the importance of conducting research during winter, a season largely neglected in ecotoxicology (Powers and Hampton, 2016; Salonen et al., 2009; Hampton et al., 2015; McMeans et al., 2020). Our findings will contribute to the recent focus of understanding winter ecology, particularly in temperate and polar regions around the world, where field work is especially challenging during that time of year.

CRediT authorship contribution statement

Chelsea Aristone: conceptualization, methodology, formal analysis, investigation, writing – original draft, writing – review & editing, visualization. **Hossein Mehdi:** conceptualization, methodology, formal analysis, investigation, writing – original draft, writing – review & editing, visualization. **Jonathan Hamilton:** investigation, writing – review & editing. **Kelly L. Bowen:** conceptualization, methodology, writing – review & editing, supervision. **Warren J.S. Currie:** conceptualization, methodology, writing – review & editing, supervision. **Karen A. Kidd:** conceptualization, methodology, writing – review & editing, supervision, funding acquisition. **Sigal Balshine:** conceptualization, methodology, writing – review & editing, supervision, project administration, funding acquisition.

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.

Acknowledgments

We are extremely grateful to the many volunteers, students, technicians, and others who have helped make this project possible. The following is a list of people who we would like to specially thank (in alphabetical order): Mark Bainbridge, Jacqueline Bikker, Jennifer Bowman, Katrina Cantera, Andrea Court, Lien Dang, Rocco Iannarelli, Samantha Lau, Kyle Mataya, Markelle Morphet, Lauren Negrazis, Bert Posedowski, Robin Rozon, Matthew Salena, Hailey Schultz, Caitlyn Synyshyn, Anittha Thayaparan, and Tys Theysmeyer. This study was supported by grants from Natural Sciences Research Council of Canada (NSERC), Royal Bank of Canada Bluewater, and City of Hamilton Water to S. Balshine and K. Kidd, and an NSERC PGS-D to H. Mehdi.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153224.

References

Bahamonde, P.A., McMaster, M.E., Servos, M.R., Martyniuk, C.J., Munkittrick, K.R., 2015. Molecular pathways associated with the intersex condition in rainbow darter (Etheostoma caeruleum) following exposures to municipal wastewater in the Grand River basin, ON, Canada. Part B. Aquat. Toxicol. 159, 302–316. https://doi.org/10.1016/j.aquatox.2014.11.022.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67. https://doi.org/10.18637/jss.v067.i01.

Bernhardt, E.S., Rosi, E.J., Gessner, M.O., 2017. Synthetic chemicals as agents of global change. Front. Ecol. Environ. https://doi.org/10.1002/fee.1450.

Birge, W.J., Black, J.A., Short, T.M., Westerman, A.G., 1989. A comparative ecological and toxicological investigation of a secondary wastewater treatment plant effluent and its receiving stream. Environ. Toxicol. Chem. 8 (5), 437–450. https://doi.org/10.1002/etc.5620080510.

Brix, H., 1994. Use of constructed wetlands in water pollution control: historical development, present status, and future perspectives. Wat. Sci. Tech. 30, 209–223. https://doi.org/10.2166/wst.1994.0413.

Brown, C.J.M., Knight, B.W., McMaster, M.E., Munkittrick, K.R., Oakes, K.D., Tetreault, G.R., Servos, M.R., 2011. The effects of tertiary treated municipal wastewater on fish communities of a small river tributary in Southern Ontario, Canada. Environ. Pollut. 159 (7), 1923–1931. https://doi.org/10.1016/j.envpol.2011.03.014.

Burdon, F.J., Munz, N.A., Reyes, M., Focks, A., Joss, A., Räsänen, K., Altermatt, F., Eggen, R.I.L., Stamm, C., 2019. Agriculture versus wastewater pollution as drivers of macroinvertebrate community structure in streams. Sci. Total Environ. 659, 1256–1265. https://doi.org/10.1016/j.scitotenv.2018.12.372.

Chadd, R., 2010. Assessment of aquatic invertebrates. In: Hurford, C., Schneider, M., Cowx, I. (Eds.), Biological Monitoring in Freshwater Habitats. Springer Netherlands, Dordrecht, pp. 63–72 https://doi.org/10.1007/978-1-4020-9278-7_7.

Chapra, S.C., Robertson, A., 1977. Great Lakes eutrophication: the effects of point source control of total phosphorus. Science 196, 1448–1450. https://doi.org/10.1126/science.196.4297.1448.

City of Hamilton, 2019. https://www.hamilton.ca/home-property-and-development/water-sewer/wastewater-collection-treatment.

Covich, A.P., Palmer, M.A., Crowl, T.A., 1999. The role of benthic invertebrate species in freshwater ecosystems: zoobenthic species influence energy flows and nutrient cycling. Bioscience 49 (2), 119–127. https://doi.org/10.2307/1313537.

Cvetkovic, M., Wei, A., Chow-Fraser, P., 2010. Relative importance of macrophyte community versus water quality variables for predicting fish assemblages in coastal wetlands of the laurentian Great Lakes. J. Great Lakes Res. 36, 64e73. https://doi.org/10.1016/j.jglr.2009.10.003.

Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107, 907–938. https://doi.org/10.1289/ehp.99107s6907.

dos Reis Oliveira, P.C., Kraak, M.H.S., Pena-Ortiz, M., van der Geest, H.G., Verdonschot, P.F.M., 2020. Responses of macroinvertebrate communities to land use specific sediment food and habitat characteristics in lowland streams. Sci. Total Environ. 703, 135060. https://doi.org/10.1016/j.scitotenv.2019.135060.

Du, S.N.N., McCallum, E.S., Vaseghi-Shanjani, M., Choi, J.A., Warriner, T.R., Balshine, S., Scott, G.R., 2018. Metabolic costs of exposure to wastewater effluent lead to compensatory adjustments in respiratory physiology in bluegill sunfish. Environ. Sci. Technol. 52, 801–811. https://doi.org/10.1021/acs.est.7b03745.

Du, S.N.N., Choi, J.A., McCallum, E.S., McLean, A.R., Borowiec, B.G., Balshine, S., Scott, G.R., 2019. Metabolic implications of exposure to wastewater effluent in bluegill sunfish. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 224, 108562. https://doi.org/10.1016/j.cbpc.2019.108562.

Englert, D., Zubrod, J.P., Schulz, R., Bundschuh, M., 2013. Effects of municipal wastewater on aquatic ecosystem structure and function in the receiving stream. Sci. Total Environ. 454–455, 401–410. https://doi.org/10.1016/j.scitotenv.2013.03.025.

Environment Canada, 2001. The State of Municipal Wastewater Effluents in Canada.

Fausch, K.D., Lyons, J., Karr, J.R., Angermeier, P.L., 1990. Fish communities as indicators of environmental degradation. Am. Fish. Soc. Symp. 8, 123e144.

Frouz, J., Matěna, J., Ali, A., 2003. Survival strategies of chironomids (Diptera: Chironomidae) living in temporary habitats: a review. European Journal of Entomology 100 (4), 459–465. https://doi.org/10.14411/eje.2003.069.

Fuzzen, M.L.M., Bennett, C.J., Tetreault, G.R., McMaster, M.E., Servos, M.R., 2015. Severe intersex is predictive of poor fertilization success in populations of rainbow darter (Etheostoma caeruleum). Aquat. Toxicol. 160, 106–116. https://doi.org/10.1016/j.aquatox.2015.01.009.

Gardarsdottir, H., Egberts, T.C.G., van Dijk, L., Heerdink, E.R., 2010. Seasonal patterns of initiating antidepressant therapy in general practice in the Netherlands during 2002–2007. J. Affect. Disord. 122, 208–212. https://doi.org/10.1016/j.jad.2009.06.033.

Gleason, J.E., Rooney, R.C., 2017. Aquatic macroinvertebrates are poor indicators of agricultural activity in northern prairie pothole wetlands. Ecol. Indic. 81, 333–339. https://doi.org/10.1016/j.ecolind.2017.06.013.

Gopal, B., 1999. Natural and constructed wetlands for wastewater treatment: potentials and problems. Wat. Sci. Tech 40, 27–35. https://doi.org/10.1016/S0273-1223(99)00468-0.

Government of Canada, 2017. Hamilton harbour: area of concern. https://www.canada.ca/en/environment-climate-change/services/great-lakes-protection/areas-concern/hamilton-harbour.html.

Grantham, T.E., Cañedo-Argüelles, M., Perrée, I., Rieradevall, M., Prat, N., 2012. A mesocosm approach for detecting stream invertebrate community responses to treated wastewater effluent. Environ. Pollut. 160, 95–102. https://doi.org/10.1016/j.envpol.2011.09.014.

Great Lakes Water Quality Agreement, 2012. https://binational.net/2012/09/05/%202012-glwqa-aqegl/.

Hamdhani, H., Eppehimer, D.E., Bogan, M.T., 2020. Release of treated effluent into streams: A global review of ecological impacts with a consideration of its potential use for environmental flows, pp. 1–14 https://doi.org/10.1111/fwb.13519.

Hamoda, M.F., Al-Ghusain, I., Al-Mutairi, N.Z., 2004. Sand filtration of wastewater for tertiary treatment and water reuse. Desalination 164 (3), 203–211. https://doi.org/10.1016/S0011-9164(04)00189-4.

Hampton, S.E., Moore, M.V., Ozersky, T., Stanley, E.H., Polashenski, C.M., Galloway, A.W.E., 2015. Heating up a cold subject: prospects for under-ice plankton research in lakes. J. Plankton Res. 37 (2), 277–284. https://doi.org/10.1093/plankt/fbv002.

Hickey, C.W., Clements, W.H., 1998. Effects of heavy metals on benthic macroinvertebrate communities in New Zealand streams. Environ. Toxicol. Chem. 17 (11), 2338–2346. https://doi.org/10.1002/etc.5620171126.

Hill, M.J., Sayer, C.D., Wood, P.J., 2016. When is the best time to sample aquatic macroinvertebrates in ponds for biodiversity assessment? Environ. Monit. Assess. 188 (3), 194. https://doi.org/10.1007/s10661-016-5178-6.

Holeton, C., Chambers, P.A., Grace, L., 2011. Wastewater release and its impacts on Canadian waters. Can. J. Fish. Aquat. Sci. 68, 1836–1859. https://doi.org/10.1139/f2011-096.

Huong, N.T.T., Duc, P.A., Mien, P.Van, 2017. Changes of benthic macroinvertebrates in thi Vai River and cai mep estuaries under polluted conditions with industrial wastewater. GeoScience Engineering 63 (2), 19–25. https://doi.org/10.1515/gse-2017-0008.

Jesus, T., Abreu, I., Guerreiro, M.J., Monteiro, A., 2020. Study of the effect of two wastewater treatment plants (WWTP's) discharges on the benthic macroinvertebrate communities' structure of the river tinto (Portugal). Limnetica 39 (1), 353–372. https://doi.org/10.23818/limn.39.23.

Jones, C., Somers, K.M., Reynoldson, T.B., 2007. Ontario benthos biomonitoring network: Protocol manual. Ontario Minstry of Environment, Sorest, ON.

Kinouchi, T., Yagi, H., Miyamoto, M., 2007. Increase in stream temperature related to anthropogenic heat input from urban wastewater. J. Hydrol. 335, 78–88. https://doi.org/10.1016/j.jhydrol.2006.11.002.
Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. Streams, 1999–2000: a national reconnaissance. Environ. Sci. Technol. 36, 1202–1211. https://doi.org/10.1021/es011055j.
Kot-Wasik, A., Jakimska, A., Śliwka-Kaszyńska, M., 2016. Occurrence and seasonal variations of 25 pharmaceutical residues in wastewater and drinking water treatment plants. Environ. Monit. Assess. 188 (12), 661. https://doi.org/10.1007/s10661-016-5637-0.
Krumhansl, K.A., Krkosek, W.H., Greenwood, M., Ragush, C., Schmidt, J., Grant, J., Barrell, J., Lu, L., Lam, B., Gagnon, G.A., Jamieson, R.C., 2015. Assessment of arctic community wastewater impacts on marine benthic invertebrates. Environ. Sci. Technol. 49 (2), 760–766. https://doi.org/10.1021/es503330n.
Lau, S.C., Mehdi, H., Bragg, L.M., Servos, M.R., Balshine, S., Scott, G.R., 2021. Exposure to wastewater effluent disrupts hypoxia responses in killifish (Fundulus heteroclitus). Environ. Pollut. 284 (November 2020), 117373. https://doi.org/10.1016/j.envpol.2021.117373.
Lawrence, G., Pieters, R., Zaremba, L., Tedford, T., Gu, L., Greco, S., Hamblin, P., 2004. Summer exchange between Hamilton harbour and Lake Ontario. Deep-Sea Research Part II: Topical Studies in Oceanography 51 (4–5), 475–487. https://doi.org/10.1016/j.dsr2.2003.09.002.
Leslie, J.K., Timmins, C.A., 1992. Distribution and abundance of larval fish in Hamilton harbour, a severely degraded embayment of Lake Ontario. J. Great Lakes Res. 18, 700e708. https://doi.org/10.1016/S0380-1330(92)71330-6.
Luo, D., Ganesh, S., Koolaard, K., 2020. predictmeans: Calculate Predicted Means for Linear Models. R package version 1.0.4. https://CRAN.R-project.org/package=predictmeans.
McCallum, E.S., Krutzelmann, E., Brodin, T., Fick, J., Sundelin, A., Balshine, S., 2017. Exposure to wastewater effluent affects fish behaviour and tissue-specific uptake of pharmaceuticals. Sci. Total Environ. 605–606, 578–588. https://doi.org/10.1016/j.scitotenv.2017.06.073.
McCallum, E.S., Nikel, K.E., Mehdi, H., Du, S.N.N., Bowman, J.E., Midwood, J.D., Kidd, K.A., Scott, G.R., Balshine, S., 2019. Municipal wastewater effluent affects fish communities: a multi-year study involving two wastewater treatment plants. Environ. Pollut. 252, 1730–1741. https://doi.org/10.1016/j.envpol.2019.06.075.
McCormick, A.R., Hoellein, T.J., London, M.G., Hittie, J., Scott, J.W., Kelly, J.J., 2016. Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages. Ecosphere 7. https://doi.org/10.1002/ecs2.1556.
McLean, A.R., Du, S.N.N., Choi, J.A., Culbert, B.M., McCallum, E.S., Scott, G.R., Balshine, S., 2019. Proximity to wastewater effluent alters behaviour in bluegill sunfish (Lepomis machrochirus). Behaviour 156, 1–23. https://doi.org/10.1163/1568539X-00003576.
McMeans, B.C., McCann, K.S., Guzzo, M.M., Bartley, T.J., Bieg, C., Blanchfield, P.J., Fernandes, T., Giacomini, H.C., Middel, T., Rennie, M.D., Ridgway, M.S., Shuter, B.J., 2020. Winter in water: differential responses and the maintenance of biodiversity. Ecol. Lett. 23, 922–938. https://doi.org/10.1111/ele.13504.
McQueen, D.J., Post, J.R., Mills, E.L., 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. 43, 1571–1581. https://doi.org/10.1139/f86-195.
Mehdi, H., Dickson, F.H., Bragg, L.M., Servos, M.R., Craig, P.M., 2018. Impacts of wastewater treatment plant effluent on energetics and stress response of rainbow darter (Etheostoma caeruleum) in the Grand River watershed. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 224, 270–279. https://doi.org/10.1016/j.cbpb.2017.11.011.
Mehdi, H., Lau, S.C., Synyshyn, C., Salena, M.G., McCallum, E.S., Muzzatti, M.N., Bowman, J.E., Mataya, K., Bragg, L.M., Servos, M.R., Kidd, K.A., Scott, G.R., Balshine, S., 2021. Municipal wastewater as an ecological trap: effects on fish communities across seasons. Sci. Total Environ., 143430 https://doi.org/10.1016/j.scitotenv.2020.143430.
Nikel, K.E., McCallum, E.S., Mehdi, H., Du, S.N.N., Bowman, J.E., Midwood, J.D., Scott, G.R., Balshine, S., 2021. Fish living near two wastewater treatment plants have unaltered thermal tolerance but show changes in organ and tissue traits. J. Great Lakes Res. https://doi.org/10.1016/j.jglr.2021.01.017.
Oksanen, J.F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan.
Ortiz, J.D., Martí, E., Puig, M.À., 2005. Recovery of the macroinvertebrate community below a wastewater treatment plant input in a Mediterranean stream. Hydrobiologia 545 (1), 289–302. https://doi.org/10.1007/s10750-005-3646-z.
Peschke, K., Capowiez, Y., Köhler, H.R., Wurm, K., Triebskorn, R., 2019. Impact of a wastewater treatment plant upgrade on amphipods and other macroinvertebrates: individual and community responses. Front. Environ. Sci. 7 (May), 1–13. https://doi.org/10.3389/fenvs.2019.00064.
Powers, S.M., Hampton, S.E., 2016. Winter limnology as a new frontier. Limnol. Oceanogr. Bull. 25 (4), 103–108. https://doi.org/10.1002/lob.10152.
R Core Team, 2021. R: a language and environment for statistical computing.
Resh, V.H., Unzicker, J.D., 1975. Water quality monitoring and aquatic organisms: the importance of species identify cation. J. Water. Pollut. Control Fed. 47, 9–19.
Saaristo, M., Myers, J., Jacques-Hamilton, R., Allinson, M., Yamamoto, A., Allinson, G., Pettigrove, V., Wong, B.B.M., 2014. Altered reproductive behaviours in male mosquitofish living downstream from a sewage treatment plant. Aquat. Toxicol. 149, 58–64. https://doi.org/10.1016/j.aquatox.2014.02.001.
Salonen, K., Leppäranta, M., Viljanen, M., Gulati, R.D., 2009. Perspectives in winter limnology: closing the annual cycle of freezing lakes. Aquat. Ecol. 43 (3), 609–616. https://doi.org/10.1007/s10452-009-9278-z.
Schindler, D.W., 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnol. Oceanogr. 23 (3), 478–486. https://doi.org/10.4319/lo.1978.23.3.0478.
Smith, L.A., Chow-Fraser, P., 2010. Impacts of adjacent land use and isolation on marsh bird communities. Environ. Manag. 45, 1040e1051. https://doi.org/10.1007/s00267-010-9475-5.
St Lawrence River Institute of Environmental Sciences, 2005. Key to Freshwater macroinvertebrates in Ontario. http://www.ap.smu.ca/~lcampbel/SLRIES_FWInverts_Ontario.pdf.
Strickland, T., Fisher, L., Korleski, C., 2010. Methods of Assessing Habitat in Lake Erie Shoreline Waters Using the Qualitative Habitat Evaluation Index (QHEI) Approach (Version 2.1).
Sui, Q., Huang, J., Deng, S., Chen, W., Yu, G., 2011. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environ. Sci. Technol. 45 (8), 3341–3348. https://doi.org/10.1021/es200248d.
Sumpter, J.P., 2009. Protecting aquatic organisms from chemicals: the harsh realities. Philos. Transact. A Math. Phys. Eng. Sci. 367, 3877–3894. https://doi.org/10.1098/rsta.2009.0106.
Taft, B., Koncelik, J.P., 2006. Methods for Assessing Habitat in Flowing Waters: Using the Qualitative Habitat Evaluation Index (QHEI).
ter Laak, T.L., van der Aa, M., Houtman, C.J., Stoks, P.G., van Wezel, A.P., 2010. Relating environmental concentrations of pharmaceuticals to consumption: a mass balance approach for the river Rhine. Environ. Int. 36, 403–409. https://doi.org/10.1016/j.envint.2010.02.009.
Ternes, T.A., Joss, A., Siegrist, H., 2004. Scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environ. Sci. Technol. 38, 392A–399A. https://doi.org/10.1021/es040639t.
Tetreault, G.R., Brown, C.J., Bennett, C.J., Oakes, K.D., McMaster, M.E., Servos, M.R., 2013. Fish community responses to multiple municipal wastewater inputs in a watershed: fish community changes near wastewater effluent outfalls. Integr. Environ. Assess. Manag. 9 (3), 456–468. https://doi.org/10.1002/ieam.1364.
Theysmeyer, T., Bowman, J., 2017. Western Desjardins Canal and West Pond conditions summary report. International report No. 2017-1. (Hamilton, Ontario).
Tuncay, E., 2016. The Effect of Wastewater Treatment Plant Effluent on Water Temperature, Macroinvertebrate Community, and Functional Feeding Groups Structure in the Lower Rouge River, Michigan. https://doi.org/10.13140/RG.2.2.19593.85600.
van der Meer, T.V., van der Lee, G.H., Verdonschot, R.C.M., Verdonschot, P.F.M., 2021. Macroinvertebrate interactions stimulate decomposition in WWTP effluent-impacted aquatic ecosystems. Aquat. Sci. 83 (4), 1–11. https://doi.org/10.1007/s00027-021-00821-8.
Vieno, N., Tuhkanen, T., Kronberg, L., 2005. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environ. Sci. Technol. 39, 8220–8226. https://doi.org/10.1021/es051124k.
Walsh, C.J., Roy, A.H., Feminella, J.W., Cottingham, P.D., Groffman, P.M., Morgan, R.P., 2005. The urban stream syndrome: current knowledge and the search for a cure. J. N. Am. Benthol. Soc. 24 (3), 706–723. https://doi.org/10.1899/04-028.1.
West Virginia Department of Environmental Protection, n.d.West Virginia Department of Environmental Protection. (n.d.). Field Guide to Aquatic Invertebrates. Retrieved August 24, 2020, from https://dep.wv.gov/WWE/getinvolved/sos/Documents/Benthic/WVSOSAdvanced_MacroGuide.pdf.
Witty, L.M., Sarrazin-Delay, C., 2014. Illustrated Guide to Boreal Shield Invertebrate Benthos, Cooperative Freshwater Ecology Unit, 2014. https://doi.org/10.13140/RG.2.2.26811.87841.
Yu, H., Song, Y., Tu, X., Du, E., Liu, R., Peng, J., 2013. Assessing removal efficiency of dissolved organic matter in wastewater treatment using fluorescence excitation emission matrices with parallel factor analysis and second derivative synchronous fluorescence. Bioresour. Technol. 144, 595–601. https://doi.org/10.1016/j.biortech.2013.07.025.
Zokan, M., Drake, J.M., 2015. The effect of hydroperiod and predation on the diversity of temporary pond zooplankton communities. Ecol. Evol. 5 (15), 3066–3074. https://doi.org/10.1002/ece3.1593.