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Municipal wastewater as an ecological trap: Effects on fish communities across seasons

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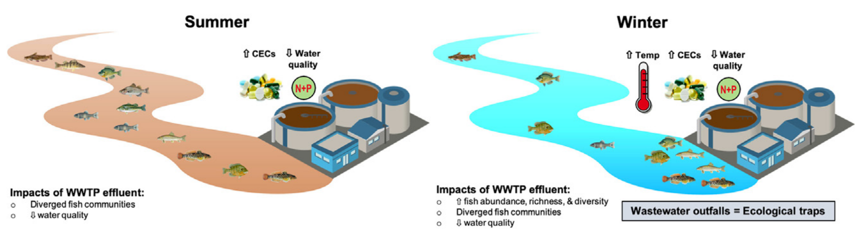
HIGHLIGHTS

- Fish communities sampled in the summer and winter along two effluent gradients
- Higher abundance, richness, and diversity near effluent outfall, only in winter
- Communities of fish closest and farthest from the outfall were most dissimilar.
- WWTP effluents impacted water quality downstream, particularly in winter.
- WWTP effluent quality (concentrations of CECs) was poorer in winter than summer.

GRAPHICAL ABSTRACT



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ABSTRACT

Municipal wastewater treatment plant (WWTP) effluents are a ubiquitous source of contamination whose impacts on fish and other aquatic organisms span across multiple levels of biological organization. Despite this, few studies have addressed the impacts of WWTP effluents on fish communities, especially during the winter—a season seldom studied. Here, we assessed the impacts of wastewater on fish community compositions and various water quality parameters during the summer and winter along two effluent gradients in Hamilton Harbour, an International Joint Commission Area of Concern in Hamilton, Canada. We found that fish abundance, species richness, and species diversity were generally highest in sites closest to the WWTP outfalls, but only significantly so in the winter. Fish community compositions differed greatly along the effluent gradients, with sites closest and farthest from the outfalls being the most dissimilar. Furthermore, the concentrations of numerous contaminants of emerging concern (CECs) in the final treated effluent were highest during the winter. Water quality of sites closer to the outfalls was poorer than at sites farther away, especially during the winter. We also demonstrated that WWTPs can significantly alter the thermal profile of effluent-receiving environments, increasing temperature by as much as ~9 °C during the winter. Our results suggest that wastewater plumes may act

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Winter

as ecological traps in winter, whereby fish are attracted to the favourable temperatures near WWTPs and are thus exposed to higher concentrations of CECs. This study highlights the importance of winter research as a key predictor in further understanding the impacts of wastewater contamination in aquatic ecosystems.

1. Introduction

Municipal wastewater treatment plant (WWTP) effluents, although usually treated, still contain a complex mixture of chemicals, including but not limited to excess nutrients, pesticides, metals, micro- and macroplastics, pharmaceuticals and personal care products (PPCPs), as well as natural and synthetic hormones (Daughton and Ternes, 1999; Kolpin et al., 2002; Ternes et al., 2004; Holeton et al., 2011; McCormick et al., 2016). As a result, effluents from WWTPs are a major source of environmental stressors in aquatic environments, accounting for the largest point source of contamination in Canada, by volume (Environment Canada, 2001; Holeton et al., 2011). Their continuous release into watersheds can significantly impair aquatic environments and ecosystems via chronic exposure of biota to contaminants of emerging concern (CECs), oxygen depletion, eutrophication, and even physical changes to habitats (e.g., alterations in flow, turbidity, and thermal properties; Holeton et al., 2011; Tetreault et al., 2011; Hamdhani et al., 2020).

The impacts of wastewater effluent exposure on aquatic organisms are observed across multiple levels of biological organization. Such impacts include endocrine disruption, represented by severe incidences of intersex, reduced androgen levels, and reduced fertilization success (Bahamonde et al., 2015; Fuzzen et al., 2015). Exposure to wastewater effluents also has metabolic and behavioural effects, demonstrated by an increase in metabolic rate (Du et al., 2018, 2019; Mehdi et al., 2018) and irregular courtship and aggressive behaviours (Saaristo et al., 2014; McCallum et al., 2017a; McLean et al., 2019). Comparatively, few studies have examined the impacts of wastewater effluent exposure at higher levels of biological organization, such as population- and community-level responses. This is surprising, given how relevant such impacts are for evaluating risks. For example, one common wastewater constituent, 17α-ethynylestradiol, has been linked to the collapse of a fathead minnow (*Pimephales promelas*) population and several ecosystem shifts in a whole-lake experiment (Kidd et al., 2007; Kidd et al., 2014). Other studies have also linked wastewater exposure to higher rates of male feminization and reduced breeding success in fishes, implying potential consequences to population sustainability (Jobling et al., 1998; Harris et al., 2011; Bahamonde et al., 2015; Fuzzen et al., 2015). Wastewater effluent can also reduce species richness and promote the dominance of tolerant and/or invasive species (Ra et al., 2007; Yeom et al., 2007; Brown et al., 2011; Tetreault et al., 2012; McCallum et al., 2019). Therefore, population and community disruptions from wastewater effluent may be extensive, but few other studies have examined fish communities in effluent-dominated environments.

In addition to the paucity of studies on fish communities, our knowledge about population- and community-level effects of WWTP effluents is derived solely from research conducted during warmer months of the year. It is unknown if and how the impacts of wastewater are exacerbated or diminished by colder temperatures. This is of vital concern for a number of reasons. First, in many temperate and polar regions of the globe, winter is a dominant season, lasting 4–8 months. Understanding the effects of WWTP effluents during such a prolonged season is of critical importance. Second, WWTPs often produce effluent of poorer quality in the winter; this is partly due to the higher incidence of human ailments that increase usage of PPCPs during the winter, and the reduced effectiveness of biological degradation of contaminants in WWTPs in colder temperatures (Vieno et al., 2005; Sui et al., 2011; Yu et al., 2013; Kot-Wasik et al., 2016). Third, wastewater effluent can be a source of thermal pollution—altering the temperature of receiving environments by as much as 5–10 °C in the winter (Environment Canada, 2001; Kinouchi et al., 2007; Mehdi et al., 2019). These changes in the thermal profile of aquatic environments may significantly alter aquatic communities, as ectothermic organisms (e.g., fish) are attracted to such thermally-enhanced environments, especially during the winter, when temperatures elsewhere may be suboptimal (Coutant, 1987; Cooke et al., 2004). The thermal plumes created by WWTPs may act as ecological traps (Schlaepfer et al., 2002; Battin, 2004) because fishes may use such environments as thermal refugia during the winter, accentuating exposure to contaminants in wastewater effluent.

In this study, we examined the influence of seasonality by assessing the impacts of wastewater effluent on fish communities near two WWTPs during the summer and winter. The two WWTPs chosen are located on the eastern- and western-most ends of Hamilton Harbour, one of 43 Areas of Concern under the Great Lakes Water Quality Agreement (Great Lakes Water Quality Agreement, 2012). Wastewater contamination is a stressor that is of particular concern in Hamilton Harbour, as it is estimated that ~50% of its inflow comes from wastewater (Government of Canada, 2017). We examined various fish community indices along a distance- and therefore contamination-gradient from the two plants to assess the impacts of wastewater exposure on the integrity of these respective ecosystems. In addition, water quality parameters and the concentrations of various PPCPs and other anthropogenic compounds in the final treated effluent were characterized to assess the abiotic impacts of wastewater contamination. We predicted that wastewater inputs would have significant effects on the physical habitat of effluent-receiving environments, thus affecting fish communities in these impacted sites. We expected this to be more apparent during the winter, when the quality of the effluent is expected to decline. If fish seek thermal refuge near WWTP outflows, then we predicted these sites would be highest in fish abundance, species richness, and species diversity, and be most compositionally distinct from sites farther away, particularly during the winter.

2. Materials and methods

2.1. Sampling sites

2.1.1. Dundas WWTP

The Dundas WWTP is located on the western tip of Cootes Paradise Marsh, the largest wetland west of Lake Ontario. The treatment facility is rated as a conventional activated sludge plant with tertiary filtration. The facility treats the majority of wastewater from the Dundas population (~30,000 people) and has a capacity of 18.2 million litres per day (City of Hamilton, 2019). The plant's effluent is discharged into the Desjardins Canal, an old shipping corridor located on the westernmost end of Cootes Paradise Marsh (Theysmeyer and Bowman, 2017). Five sites, varying in distance from the effluent outflow, were sampled in 2018 and 2019. Site selection was based on a previous study (McCallum et al., 2019) and accessibility in both summer and winter. Of the sites sampled, three were in the direct flow path of the effluent discharged from the WWTP: D1 was immediately adjacent to the outflow, and D2 and D3 were 550 and 1000 m downstream, respectively (Fig. 1A). In addition, two reference sites were also sampled: D4, located at the mouth of Spencer Creek which was 2800 m downstream, and D5, located at the southwestern edge of Cootes Paradise Marsh which was 3750 m downstream; neither reference site was in the direct flow path of the wastewater effluent (Fig. 1A). All sites were sampled four times in the summer (July to August) and three times in the winter (November to March).

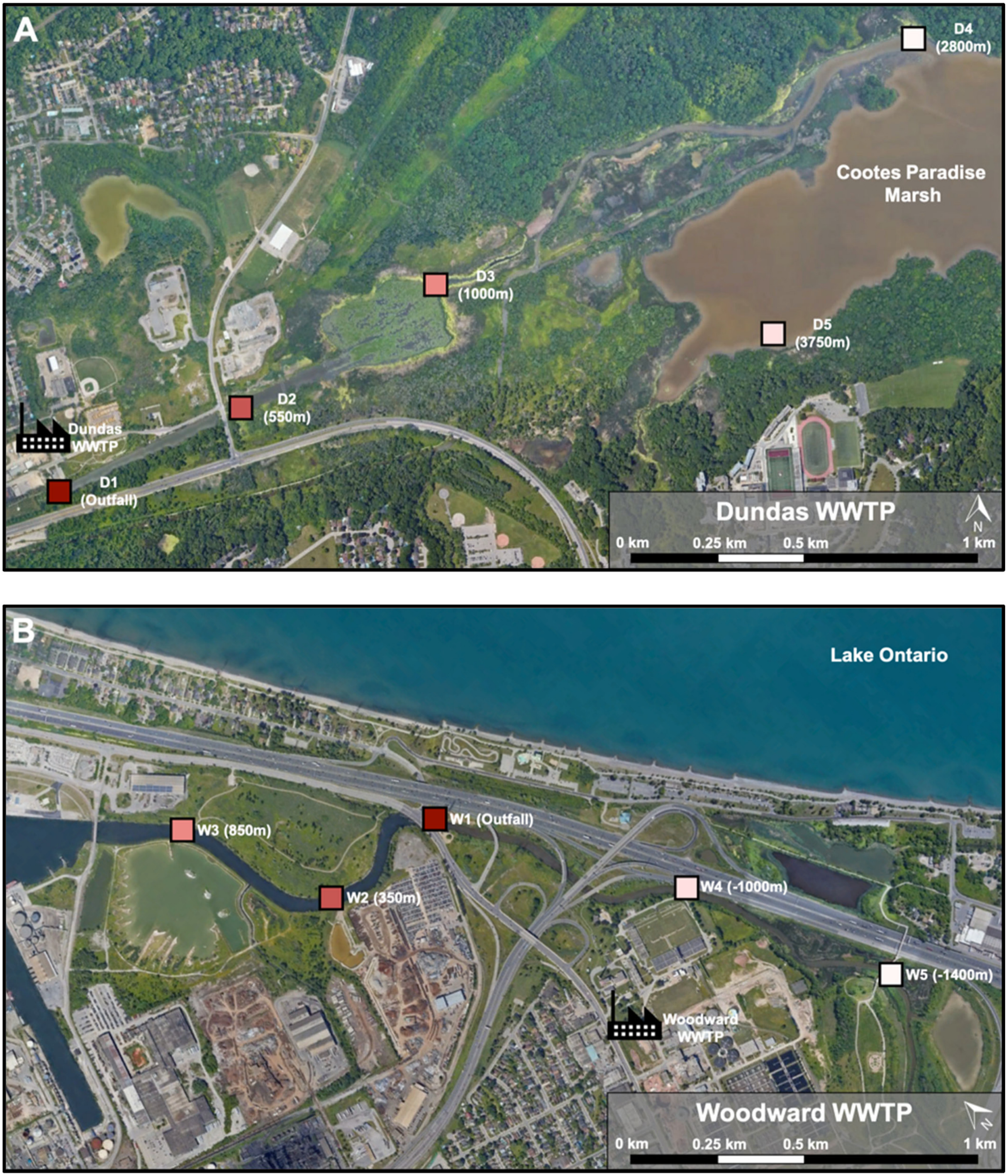

Fig. 1. Map of our sampling sites along a distance gradient from the (A) Dundas and (B) Woodward WWTPs. The location of each WWTP is also displayed. Maps generated in Google Earth Pro 7.3.2.5776, imagery date 06/30/2018 and accessed on 24/02/2020.

2.1.2. Woodward WWTP

The Woodward WWTP is located on the southeastern corner of Hamilton Harbour, ON, Canada. The Woodward WWTP is the largest WWTP in Hamilton, serving ~480,000 people and has an average daily capacity of 409 million litres (City of Hamilton, 2019). The facility is a secondary conventional activated sludge plant with sludge dewatering and digestion (City of Hamilton, 2019). Effluent from the plant is released into Red Hill Creek which flows into Hamilton Harbour. Similar to Dundas, five sites were sampled in 2018 and 2019 (Fig. 1B). Three sites were in the direct flow path of the effluent: W1 which was immediately adjacent to the outflow, and W2 and W3, which were 350 and 850 m downstream, respectively. Two upstream reference sites were also sampled: W4 and W5 which were 1000 and 1400 m upstream, respectively. Similar to the Dundas WWTP, sites were selected based on accessibility during both seasons. All sites were sampled five times in the summer (July to August) and three times in the winter (December to March).

2.2. Fish sampling

Fish communities were surveyed both in the summer and winter on weather permitting days (i.e., days free of storms and ice cover), using passive (minnow and Windermere traps) and active (electrofishing from a boat) sampling gear. At each site, 10 minnow traps, baited with ~20 g of corn, were set ~10 m apart from each other and deployed from land. Two Windermere traps, each baited with ~100 g of corn, flanking the first and last minnow traps, were also deployed. Minnow and Windermere traps were retrieved 24 h post-deployment. In addition, two 50 m-transects were electrofished at each site (1.5-KVA Electrofisher, Smith-Root Inc.). All fish caught were transported in dark-coloured bins with aerators to a field site where they were identified to species level, measured (total and standard lengths), and weighed. To speed up processing and reduce handling stress, the first 15 individuals of a given species, sampling technique, and site were individually measured, while the remaining individuals caught of that species, site, and sampling technique were identified to species level, counted, and then batch-weighed. Native fish were immediately released back to their site of collection, while invasive fish were euthanized with an overdose of benzocaine (small fishes) or by a lethal cephalic blow (large fishes), as required by the Ontario Ministry of Natural Resources (2015). All fish were handled in accordance with approved animal use protocols from McMaster University's Animal Research Ethics Board (AUP 17-12-45).

2.3. Water quality, chemistry, and habitat characterization

At each site, the following water quality parameters were measured simultaneously to fish sampling: temperature and dissolved oxygen (YSI ProODO), pH, conductivity, total dissolved solids, and salinity (Oakton multiparameter Testr). Furthermore, 1 L water samples were collected from the middle of the water column at each site using a 2.2 L Van Dorn sampler. Water samples were analyzed for ammonia + ammonium, nitrate, nitrite, o-phosphate, total phosphorus, and total Kjeldahl nitrogen by the City of Hamilton Environmental Laboratory using methods previously described in McCallum et al. (2019). Long-term water temperature was also measured for 14 days by deploying temperature data loggers (HOBO Pendant MX Temp) at the outfall sites and one of the reference sites (D4 and W4) for both WWTPs during the summer (September 2018) and winter (December 2019).

Additionally, 24 h composite samples of final effluent were collected from both the Dundas and Woodward WWTPs twice a week during the months of August 2019 (summer; $n_{Dundas} = 7$, $n_{Woodward} = 8$) and December 2019 (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 City of Hamilton Environment Laboratory, see Supplementary Table 1 for these values). Composite effluent samples were further analyzed for a wide range of PPCPs and other CECs using already established methods by Arlos et al. (2015). Briefly, solid phase extraction was used to concentrate the compounds found in the composite effluent samples into 500 μL extracts. Extracts were then analyzed using an Agilent 1260 HPLC with 6460 triple quad mass spectrometer (LC-MS/MS) with Agilent Jet Stream (AJS) electrospray ionization in both positive and negative modes. Twenty-one different compounds of nine different classes were analyzed: lipid regulators, antiepileptics, analgesics, stimulants, antibacterials, antibiotics, antidepressants, non-steroidal anti-inflammatory agents (NSAIDs), and herbicides. Detailed analysis parameters are described in Supplementary Tables 2–5.

The habitat characteristics of each sampling site were assessed following protocols developed in a previous study (McCallum et al., 2019) and were based on a subset of metrics used in the Qualitative Habitat Evaluation Index (Taft and Koncelik, 2006; Strickland et al., 2010). The following parameters were assessed: total 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 Tables 6 and 7).

2.4. Statistical analyses

All statistical analyses were conducted using R (version 3.6.2, R Core Team, 2019). Prior to any analysis of fish communities, counts of species were mean-standardized (multi-gear mean standardization) following methods outlined in Gibson-Reinemer et al. (2017) and McCallum et al. (2019), as this allowed for data from different gear types to be analyzed together. Catch per unit effort (CPUE) was calculated on a per-trap deployed basis for minnow and Windermere traps and per-second shocked for electrofishing. All species, rare and common, were included in the final analyses. Fish abundance, species richness, and effective species diversity (Shannon-Wiener Index) were analyzed using permutation linear mixed effects models (PLMM) with 5000 iterations using the lme4 and predictmeans packages (Bates et al., 2015; Luo et al., 2020). These response variables were analyzed using sampling site (as a numeric factor representing both distance from outfall and contamination load) and season as main effects, and sampling period within each season as a random effect. Principal coordinate analysis (PCoA) was then performed using a Bray-Curtis dissimilarity matrix to assess fish community composition differences between sites and seasons. Beta diversity was then visually explored using PCoA biplots, with site-specific 80% confidence ellipses overlaid to delineate community composition differences (Oksanen et al., 2019). Fish community composition differences between sites and seasons were further analyzed with a permutation ANOVA with 5000 permutations using adonis2 (Vegan package; Oksanen et al., 2019). Furthermore, similarity percentages (SIMPER) analysis was used to identify which key species were driving the differences observed in the community assemblages between the outfall sites and all other sampling sites. While all species were included in the analysis, only those that contributed ≥5% of the total abundance were further interpreted. Permutation tests ($n = 5000$) were used to identify between-site significant differences in CPUE of key species. Indicator species analysis (De Cáceres and Legendre, 2009) was then used to identify which species were representative or indicative of sites closest (D1-D2 and W1-W2) and farthest (D4-D5 and W4-W5) from the outfall in both seasons. Permutation tests ($n = 5000$) were carried out to determine the significance of these particular species as indicators.

To reduce the number of analyses needed and for ease of visualization, water quality parameters were plotted and analyzed collectively using principal component analysis (PCA). Overall sampling site differences in water quality parameters, as expressed by the first two principal components, were analyzed using a permutation ANOVA with 5000 permutations using adonis2 (Vegan package; Oksanen et al., 2019). Differences in individual water quality parameters were tested as a function of site and season using a permutation MANOVA with 5000 iterations. Long-term water temperature data were analyzed using linear models (LM) to test for site differences within each season. Finally, a permutation MANOVA was used to analyze the chemical makeup of the composite effluent samples. All 21 compounds that were measured to characterize the effluent were analyzed simultaneously to test for seasonal differences (lmPerm, Wheeler and Torchiano, 2016). For statistical purposes, compounds that were below the detection limit were analyzed as zeros. All analyses were conducted separately between each WWTP. Data are reported as means ± standard error (SE) unless otherwise stated, and in all analyses, a difference was deemed significant when $p < 0.05$.

3. Results

3.1. Fish community metrics

3.1.1. Dundas WWTP

At the Dundas WWTP sampling sites, 2388 fish were collected (2112 in the summer and 276 in the winter) consisting of 23 unique species (Supplementary Table 8). Overall, fish were less abundant (based on CPUE) in the winter than in the summer (PLMM, $t = -4.63$, $p < 0.01$; Fig. 2A). In the winter, abundance declined with distance from the outfall ($t_{(\text{Winter})} = -3.13$, $p < 0.01$; Fig. 2A); however, no such decline was observed in the summer ($t_{(\text{Summer})} = 0.83$, $p = 0.39$; Fig. 2A). Similarly, species richness was greatly reduced in the winter ($t = -4.08$, $p < 0.001$; Fig. 2B). As with abundance in the winter, sites closer to the outfall were more species-rich than those farther away ($t_{(\text{Winter})} = 3.95$, $p < 0.001$; Fig. 2B); however, in the summer, species richness was not linked to proximity to the outfall ($t_{(\text{Summer})} = 0.52$, $p = 0.58$; Fig. 2B). Effective species diversity (Shannon-Wiener index) was lower in the winter than in the summer ($t = -4.39$, $p < 0.01$), and declined with distance from the outfall during the winter ($t_{(\text{Winter})} = -3.24$, $p < 0.01$) but not in the summer ($t_{(\text{Summer})} = -1.31$, $p = 0.65$). The tolerance, resilience, trophic level, and the proportion of non-native to native species of fishes caught across sites and seasons are described in the Supplementary Materials.

Fish community composition differed significantly between seasons (Permutation ANOVA, $F_{(\text{Season})} = 3.33$, $p < 0.001$; Fig. 3A and B). In the summer, fish community compositions differed significantly across sites ($F_{(\text{Summer})} = 3.13$, $p < 0.001$; Fig. 3A). Communities at sites closest to the outfall were most different from those farthest away, while there was considerable overlap in the intermediate sites (PCoA; Fig. 3A). In the winter, fish communities were separated in a manner similar to in the summer, where sites closer to the outfall were most different than those farther away ($F_{(\text{Winter})} = 2.18$, $p < 0.001$; Fig. 3B). Similarity analysis further highlighted which species were driving the community changes across sites (Table 1). In the summer, fish community differences were largely driven by round goby being consistently more abundant in D1 than in all other sites. Similarly, white sucker were more abundant in D1 than all other sites except D5. Green sunfish were more abundant in D1 than D3. Relative to D1, largemouth bass, yellow perch, and goldfish were more abundant in D2, D4, and D5, respectively. During the winter, fewer species appeared to be driving across-site community differences compared to during the summer. Round goby were again more abundant in D1 than in D2 and D3. Bluegill sunfish were more abundant in D1 compared to D2, while common logperch were more abundant in D4 compared to D1. See Supplementary Table 9 for further details on all site and season comparisons. Several indicator species were identified using indicator species analysis. During the summer, sites closer to the outfall were mostly identifiable by the presence of white sucker ($p < 0.01$) and round goby ($p < 0.001$), while sites farther away were more identifiable by the presence of bluegill sunfish ($p = 0.02$) and white perch ($p < 0.001$). During the winter, sites closer the outfall tended to be more identifiable by round goby ($p = 0.05$), green sunfish ($p = 0.05$) and largemouth bass ($p = 0.06$), while sites farther away were more identifiable by yellow perch ($p < 0.01$) and common logperch ($p < 0.001$).

3.1.2. Woodward WWTP

At the Woodward WWTP, across all sampling events, 1844 fish were caught (1546 in the summer and 298 in the winter) composed of 26 unique species (Supplementary Table 8). Overall, fish abundance was

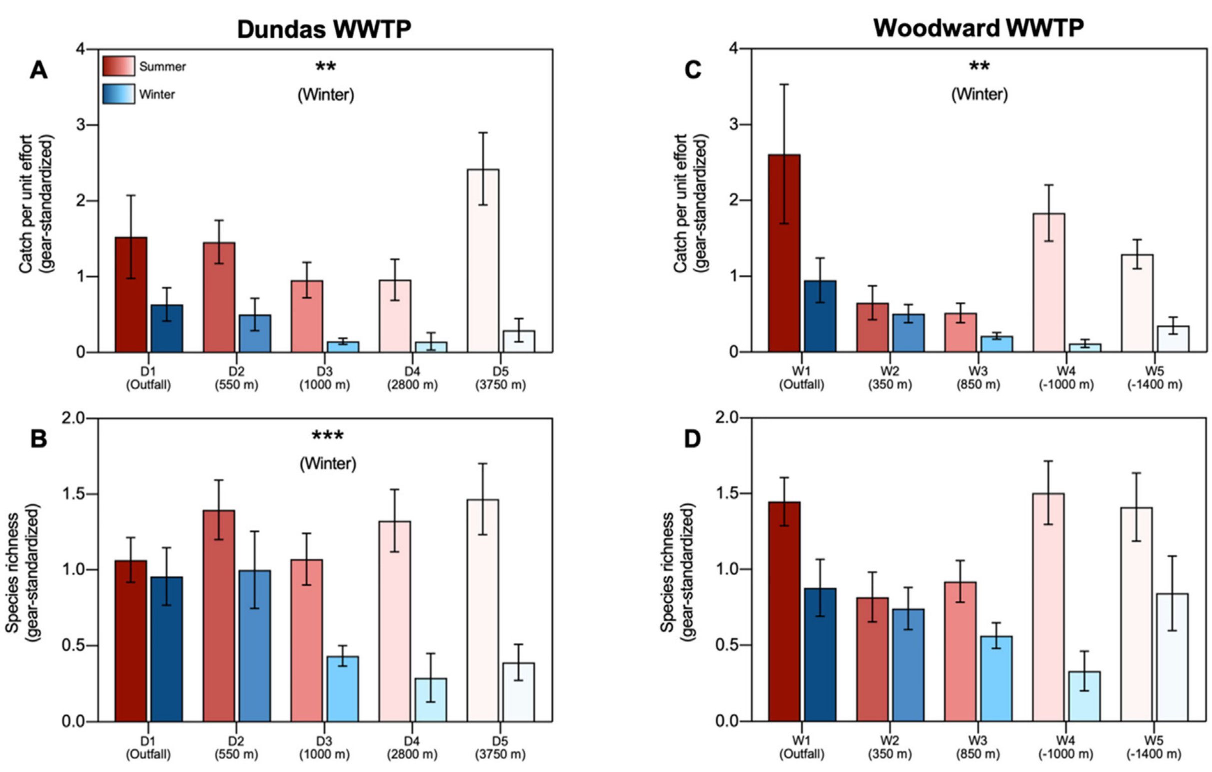

Fig. 2. Mean (±SE) gear-standardized (A, C) abundance (catch per unit effort) and (B, D) species richness, of fish caught along the effluent gradient from the Dundas and Woodward WWTP. In the winter, a significant effect of proximity to the outfall is indicated by (**$p < 0.01$) and (***$p < 0.001$). No significant trends were observed in the summer.

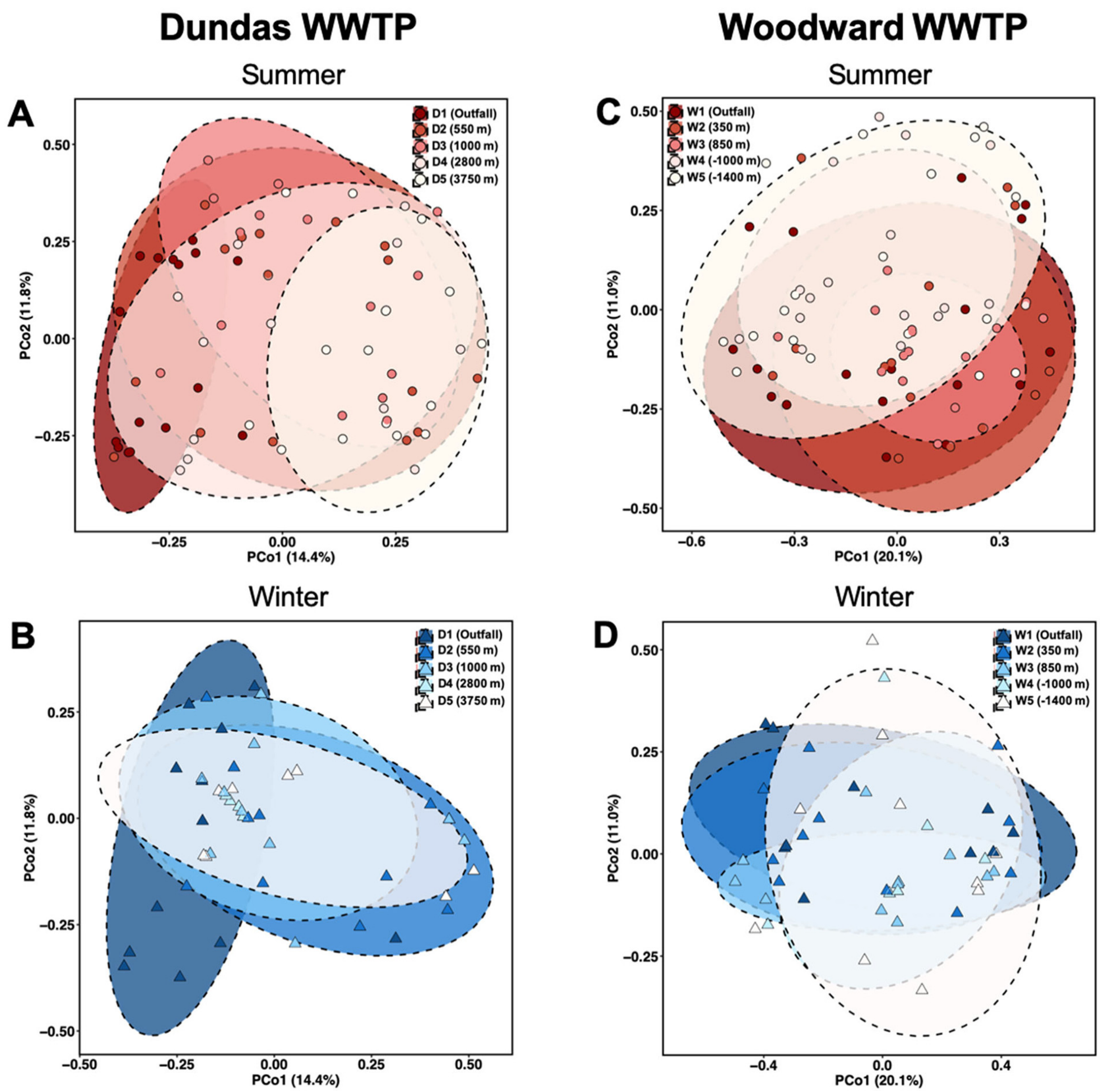

Fig. 3. Principal coordinate analysis (PCoA) ordination output of fish community compositions with 80% confidence ellipses overlaid on each site. (A, B) PCoA biplots from the Dundas WWTP in summer and winter, respectively. (C, D) PCoA biplots from the Woodward WWTP in summer and winter, respectively.

greater in summer than in winter ($t = 2.51$, $p = 0.049$; Fig. 2C). In the winter, fish abundance was highest at the outfall site and decreased with distance ($t_{(\mathrm{Winter})} = -3.08$, $p = 0.01$; Fig. 2C). However, in the summer, abundance was not related to distance from the outfall ($t_{(\mathrm{Summer})} = 0.001$, $p = 0.91$; Fig. 2C). Species richness was greater in summer compared to in winter ($t = 2.72$, $p = 0.03$; Fig. 2D) but was not correlated to proximity of the effluent outfall in either season ($t_{(\mathrm{Summer})} = 1.05$, $p = 0.29$; $t_{(\mathrm{Winter})} = 1.27$, $p = 0.22$; Fig. 2D). Effective species diversity did not show a seasonal pattern ($t = 1.52$, $p = 0.18$), nor was it related to proximity of the outfall in either season ($t_{(\mathrm{Summer})} = 0.41$, $p = 0.69$; $t_{(\mathrm{Winter})} = -0.58$, $p = 0.55$). The tolerance, resilience, trophic level, and the proportion of non-native to native species of fish caught across sites and seasons are described in the Supplementary Materials.

Fish community composition also varied significantly between seasons at the Woodward WWTP sampling sites (Permutation ANOVA, $F = 1.79$, $p < 0.001$; Fig. 3C and D). Fish communities differed significantly across sites in the summer ($F_{(\mathrm{Summer})} = 1.97$, $p < 0.001$ Fig. 3C), but not in the winter ($F_{(\mathrm{Winter})} = 1.04$, $p = 0.40$; Fig. 3D). While there was significant overlap in the PCoA output in both seasons, separation of communities was more apparent in the summer, with sites closest and farthest from the outfall being the most dissimilar (Fig. 3C and D). Community assemblages were further examined using SIMPER analysis to investigate which species were driving the changes in composition (Table 1). During the summer, brook stickleback were more abundant in W1 than in W2 and W3. Spottail shiner were more abundant in W1 than in W3, while W3 had more white sucker than W1. During the winter, cross-site community differences were absent, apart from round goby being more abundant in W1 than in W3. See Supplementary Table 10 for further details on all site and season comparisons. During the summer, indicator species analysis revealed that sites closer to the outfall were only significantly identifiable by brook stickleback ($p < 0.001$), while sites farther away were more identifiable by common carp ($p = 0.02$) and pumpkinseed sunfish ($p < 0.01$). During the winter, only gizzard shad ($p = 0.01$) were identifiable of sites closer to the outfall, while no indicator species were detected elsewhere.

Table 1

Similarity percentages (SIMPER) analysis showing the contribution of key species to the overall dissimilarity of the outfall site relative to all other sampling sites. Average A and Average B represent the gear-standardized catch per unit effort (abundance) for each species at the pair of sites being compared. Only species that contributed ≥5% to the overall abundance are shown. Bolded averages indicate significant differences ($p < 0.05$). See Supplementary Tables 9 and 10 for further details on all site and season comparisons.

Comparison	Summer					Winter				
	Total dissimilarity	Species	Average A	Average B	Contribution	Total dissimilarity	Species	Average A	Average B	Contribution
Dundas WWTP										
A. D1 (Outfall)	93.01%	*Neogobius melanostomus* (Round goby)	**1.39**	**0.09**	**27.0**	95.51%	*Neogobius melanostomus* (Round goby)	**0.46**	**0.01**	**20.0**
B. D2 (550 m)		*Ameiurus nebulosus* (Brown bullhead)	0.0004	0.86	13.8		*Ameiurus nebulosus* (Brown bullhead)	0.04	0.13	17.2
		Micropterus salmoides (Largemouth bass)	**0.32**	**0.46**	**10.2**		*Lepomis cyanellus* (Green sunfish)	0.14	0.05	12.9
		Carassius auratus (Goldfish)	0.001	0.3	9.67		*Lepomis macrochirus* (Bluegill sunfish)	**0.15**	**0.11**	**11.1**
		Scardinius erythrophthalmus (Rudd)	0.07	0.3	8.82		*Dorosoma cepedianum* (Gizzard shad)	0	0.89	6.86
		Lepomis cyanellus (Green sunfish)	0.16	0.03	7.26		*Scardinius erythrophthalmus* (Rudd)	0	0.05	6.69
		Catostomus commersonii (White sucker)	**0.59**	**0.001**	**5.43**					
A. D1 (Outfall)	95.65%	*Neogobius melanostomus* (Round goby)	**1.39**	**0**	**25.7**	97.79%	*Neogobius melanostomus* (Round goby)	**0.46**	**0**	**21.2**
B. D3 (1000 m)		*Carassius auratus* (Goldfish)	0.001	0.68	13.1		*Ameiurus nebulosus* (Brown bullhead)	0.04	0.03	10.8
		Ameiurus nebulosus (Brown bullhead)	0.0004	0.63	9.28		*Lepomis macrochirus* (Bluegill sunfish)	0.15	0.05	10.6
		Lepomis cyanellus (Green sunfish)	**0.16**	**0.01**	**8.41**		*Scardinius erythrophthalmus* (Rudd)	0	0.16	10.2
		Catostomus commersonii (White sucker)	**0.59**	**0.04**	**7.10**		*Lepomis cyanellus* (Green sunfish)	0.14	0	9.07
		Dorosoma cepedianum (Gizzard shad)	0	0.05	6.40		*Pimephales promelas* (Fathead minnow)	0.01	0.05	7.41
		Micropterus salmoides (Largemouth bass)	0.32	0.18	5.96					
		Perca flavescens (Yellow perch)	0.06	0.09	5.16					
A. D1 (Outfall)	94.53%	*Neogobius melanostomus* (Round goby)	**1.39**	**0.04**	**25.7**	97.50%	*Percina caprodes* (Common logperch)	**0.003**	**0.15**	**27.6**
B. D4 (2800 m)		*Perca flavescens* (Yellow perch)	**0.06**	**0.12**	**10.7**		*Neogobius melanostomus* (Round goby)	0.46	0	22.4
		Lepomis macrochirus (Bluegill sunfish)	0.02	0.33	10.6		*Lepomis macrochirus* (Bluegill sunfish)	0.15	0	9.82
		Ameiurus nebulosus (Brown bullhead)	0.0003	0.17	7.98		*Lepomis cyanellus* (Green sunfish)	0.14	0	9.11
		Lepomis cyanellus (Green sunfish)	0.16	0.03	6.71		*Amia calva* (Bowfin)	0	0.02	6.82
		Catostomus commersonii (White sucker)	**0.59**	**0.01**	**6.31**					
		Scardinius erythrophthalmus (Rudd)	0.07	0.15	6.21					
A. D1 (Outfall)	97.93%	*Neogobius melanostomus* (Round goby)	**1.39**	**0.01**	**20.0**	98.97%	*Perca flavescens* (Yellow perch)	**0**	**0.32**	**24.4**
B. D5 (3750 m)		*Ameiurus nebulosus* (Brown bullhead)	0.0003	0.96	16.5		*Ameiurus nebulosus* (Brown bullhead)	0.04	0.34	19.6
		Lepomis macrochirus (Bluegill sunfish)	0.002	3.24	16.2		*Neogobius melanostomus* (Round goby)	0.46	0	19.4
		Carassius auratus (Goldfish)	0.001	0.36	9.37		*Lepomis macrochirus* (Bluegill sunfish)	0.15	0.001	9.47
		Morone americana (White perch)	**0**	**0.93**	**8.44**		*Lepomis cyanellus* (Green sunfish)	0.14	0	8.08
		Scardinius erythrophthalmus (Rudd)	0.07	0.22	7.59					
		Lepomis cyanellus (Green sunfish)	0.16	0.12	5.45					
Woodward WWTP										
A. W1 (Outfall)	89.24%	*Culaea inconstans* (Brook stickleback)	**0.70**	**0.36**	**20.1**	83.50%	*Neogobius melanostomus* (Round goby)	0.60	0.31	26.2
B. W2 (350 m)		*Neogobius melanostomus* (Round goby)	0.68	0.13	18.5		*Lepomis cyanellus* (Green sunfish)	0.55	0.14	21.4
		Lepomis cyanellus (Green sunfish)	0.05	0.18	10.9		*Lepomis macrochirus* (Bluegill sunfish)	0.32	0.23	11.6
		Pimephales promelas (Fathead minnow)	1.62	0.08	8.31		*Lepomis gibbosus* (Pumpkinseed)	0.06	0.20	7.31

(continued on next page)

Table 1 (*continued*)

Comparison	Summer					Winter				
	Total dissimilarity	Species	Average A	Average B	Contribution	Total dissimilarity	Species	Average A	Average B	Contribution
		Catostomus commersonii (White sucker)	0.03	0.02	6.57		*Ameiurus nebulosus* (Brown bullhead)	0.06	0.23	5.49
		Lepomis macrochirus (Bluegill sunfish)	0.06	0.02	5.36					
		Notropis hudsonius (Spottail shiner)	3.55	0	5.22					
A. W1 (Outfall) B. W3 (850 m)	93.07%	*Culaea inconstans* (Brook stickleback)	**0.70**	**0.18**	**17.5**	89.73%	*Neogobius melanostomus* (Round goby)	**0.60**	**0.20**	**29.9**
		Neogobius melanostomus (Round goby)	0.68	0.01	17.4		*Lepomis cyanellus* (Green sunfish)	0.55	0.10	24.8
		Lepomis cyanellus (Green sunfish)	0.05	0.72	15.3		*Lepomis macrochirus* (Bluegill sunfish)	0.32	0.0004	10.2
		Catostomus commersonii (White sucker)	**0.03**	**0.08**	**8.63**		*Culaea inconstans* (Brook stickleback)	0	0.06	7.11
		Pimephales promelas (Fathead minnow)	1.62	0.24	8.04					
		Notropis hudsonius (Spottail shiner)	**3.55**	**0.15**	**6.6**					
		Carassius auratus (Goldfish)	0.0003	0.23	5.12					
A. W1 (Outfall) B. W4 (−1000 m)	90.40%	*Neogobius melanostomus* (Round goby)	0.68	0.87	21.6	90.23%	*Lepomis cyanellus* (Green sunfish)	0.55	0.20	26.3
		Lepomis macrochirus (Bluegill sunfish)	0.06	0.47	13.9		*Neogobius melanostomus* (Round goby)	0.60	0.08	23.0
		Culaea inconstans (Brook stickleback)	0.70	0	11.7		*Lepomis macrochirus* (Bluegill sunfish)	0.32	0.17	12.9
		Pimephales promelas (Fathead minnow)	1.62	0.17	10.3		*Notropis atherinoides* (Emerald shiner)	0.10	0.02	6.25
		Lepomis cyanellus (Green sunfish)	0.05	0.23	6.7					
		Lepomis gibbosus (Pumpkinseed)	0	0.57	6.21					
		Notropis hudsonius (Spottail shiner)	3.55	0.06	5.26					
A. W1 (Outfall) B. W5 (−1400 m)	89.03%	*Neogobius melanostomus* (Round goby)	0.68	0.41	23.8	90.10%	*Neogobius melanostomus* (Round goby)	0.60	0.12	23.9
		Culaea inconstans (Brook stickleback)	0.70	0	11.8		*Lepomis cyanellus* (Green sunfish)	0.55	0.05	20.3
		Lepomis macrochirus (Bluegill sunfish)	0.06	0.30	11.2		*Lepomis macrochirus* (Bluegill sunfish)	0.32	0.08	15.8
		Lepomis cyanellus (Green sunfish)	0.05	0.37	10.2		*Pimephales promelas* (Fathead minnow)	0.15	0.17	10.6
		Pimephales promelas (Fathead minnow)	1.62	0.13	8.2		*Lepomis gibbosus* (Pumpkinseed)	0.06	0.08	5.72
		Notropis hudsonius (Spottail shiner)	3.55	0.08	5.51					

3.2. Water quality parameters and habitat characteristics

3.2.1. Dundas WWTP

Water quality parameters varied along the effluent gradient in both seasons (Permutation ANOVA, $F_{(\mathrm{Summer})} = 3.58$, $p < 0.001$, $F_{(\mathrm{Winter})} = 5.05$, $p < 0.001$; Table 2; Fig. 4A and B; see Supplementary Table 11 for PCA loadings). Water quality differences were most apparent between sites closer to the outfall and those farthest away in both seasons (PCA; Fig. 4A and B). Overall, water temperature, soluble reactive phosphorus, and total phosphorus were higher in the summer; in contrast, pH, conductivity, salinity, total dissolved solids, total ammonia nitrogen, and total nitrate nitrogen were higher in the winter. In the summer, conductivity, total dissolved solids, salinity, total nitrate nitrogen, and total nitrogen decreased with distance from the outfall, while water temperature, pH, and soluble reactive phosphorous increased with distance from the outfall. In the winter, water temperature, conductivity, salinity, total dissolved solids, total ammonia nitrogen ($p = 0.06$), total nitrate nitrogen, total nitrogen, and total phosphorus decreased with distance from the outfall, while dissolved oxygen, and pH increased with distance from the outfall (all contrasts were $p < 0.05$, unless otherwise stated). During the summer, longer-term temperature data were not different between the outfall (D1) and reference (D4) sites (Linear Model, LM, $t_{(\mathrm{Summer})} = 1.49$; $p = 0.15$; Fig. 5A), while in the winter, there was a striking difference between the two sites ($t_{(\mathrm{Winter})} = 25.25$; $p < 0.001$; Fig. 5B), with the outfall site being on average ~ 8 °C warmer than the reference site. See Table 2 for further details on all water quality parameters.

The two sites closest to the outfall (D1 and D2) were the most anthropogenically-disturbed. These sites have been heavily impacted with clear modifications to the shoreline and are in close proximity to urban structures. In comparison, sites D3 – D5 appeared to be less disturbed with little to no human modifications. These sites are surrounded with wetland habitats and natural forests, and are farther away from urban structures. Further habitat characteristics are presented in Supplementary Table 6.

3.2.2. Woodward WWTP

Water quality parameters varied greatly between the upstream and downstream sites of the Woodward WWTP outflow during both seasons (Permutation ANOVA, $F_{(\mathrm{Summer})} = 2.51$, $p < 0.01$; $F_{(\mathrm{Winter})} = 3.17$, $p = 0.01$; Table 2; Fig. 4C and D; see Supplementary Table 11 for PCA loadings). Reduction of water quality parameters into the first

Table 2
Mean (±SE) of water quality parameters taken at each sampling site across both seasons (summer | winter). The following water quality parameters were measured: 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).

	Dundas WWTP				
	D1 (Outfall)	D2 (550 m)	D3 (1000 m)	D4 (2800 m)	D5 (3750 m)
Water temperature (°C)	21.1 ± 0.679 | 9.97 ± 0.578	23.3 ± 0.578 | 7.67 ± 0.689	24.2 ± 0.702 | 4.87 ± 1.24	23.7 ± 1.12 | 3.23 ± 0.667	26.7 ± 1.22 | 3.60 ± 1.56
DO (%)	124 ± 6.73 | 91.1 ± 4.99	134 ± 12.0 | 98.2 ± 5.54	90.7 ± 17.3 | 110. ± 1.63	87.0 ± 7.64 | 101 ± 1.54	132 ± 24.3 | 108 ± 2.41
pH	7.57 ± 0.114 | 7.69 ± 0.223	7.88 ± 0.271 | 7.84 ± 0.194	7.49 ± 0.229 | 8.40 ± 0.240	8.22 ± 0.147 | 8.78 ± 0.211	8.34 ± 0.272 | 8.80 ± 0.163
Conductivity (μS)	1190 ± 45.9 | 1450 ± 75.1	1150 ± 46.6 | 1530 ± 92.1	1130 ± 74.7 | 1490 ± 82.2	944 ± 101 | 942 ± 90.7	886 ± 64.8 | 967 ± 98.0
TDS (ppm)	845 ± 34.1 | 1010 ± 47.4	819 ± 32.0 | 1090 ± 64.7	807 ± 82.9 | 1070 ± 58.7	670. ± 70.8 | 670. ± 65.3	626 ± 45.7 | 690. ± 70.1
Salinity (ppm)	563 ± 29.3 | 719 ± 34.6	544 ± 20.7 | 756 ± 38.1	537 ± 30.8 | 732 ± 28.7	441 ± 44.6 | 462 ± 59.3	414 ± 27.0 | 476 ± 60.4
TAN (mg/L)	0.030 ± 0.006 | 0.507 ± 0.437	0.046 ± 0.011 | 0.223 ± 0.159	0.060 ± 0.020 | 0.137 ± 0.058	0.060 ± 0.039 | 0.043 ± 0.015	0 ± 0 | 0.020 ± 0.010
TNN (mg/L)	15.4 ± 0.766 | 17.1 ± 1.17	11.3 ± 1.82 | 16.1 ± 1.02	4.84 ± 1.57 | 11.6 ± 1.29	0.493 ± 0.314 | 1.07 ± 0.195	0 ± 0 | 0.690 ± 0.205
TN (mg/L)	16.3 ± 0.515 | 18.5 ± 0.695	12.5 ± 1.68 | 17.0 ± 1.03	6.43 ± 1.52 | 13.1 ± 0.973	1.53 ± 0.398 | 1.64 ± 0.229	1.38 ± 0.222 | 1.21 ± 0.252
SRP (mg/L)	0 ± 0 | 0 ± 0	0 ± 0 | 0 ± 0	0.013 ± 0.013 | 0 ± 0	0.030 ± 0.018 | 0 ± 0	0.04 ± 0.002 | 0 ± 0
TP (mg/L)	0.117 ± 0.020 | 0.079 ± 0.010	0.117 ± 0.020 | 0.079 ± 0.015	0.183 ± 0.034 | 0.071 ± 0.003	0.118 ± 0.007 | 0.065 ± 0.019	0.194 ± 0.054 | 0.052 ± 0.003
	Woodward WWTP				
	W1 (Outfall)	W2 (350 m)	W3 (850 m)	W4 (−1000 m)	W5 (−1400 m)
Water temperature (°C)	21.7 ± 0.371 | 11.3 ± 0.954	22.2 ± 0.250 | 10.7 ± 0.832	22.7 ± 0.425 | 9.77 ± 0.291	23.3 ± 0.473 | 4.13 ± 1.07	23.3 ± 1.28 | 4.43 ± 1.08
DO (%)	74.2 ± 2.60 | 69.0 ± 1.26	69.4 ± 4.47 | 68.9 ± 0.666	63.3 ± 4.10 | 67.3 ± 2.87	75.4 ± 24.5 | 92.9 ± 2.53	93.6 ± 13.0 | 95.6 ± 0.088
pH	7.07 ± 0.106 | 7.77 ± 0.231	7.08 ± 0.128 | 7.40 ± 0.091	7.06 ± 0.105 | 7.55 ± 0.091	8.05 ± 0.231 | 8.66 ± 0.427	8.13 ± 0.189 | 8.71 ± 0.317
Conductivity (μS)	1170 ± 43.5 | 2020 ± 361	1150 ± 53.2 | 2030 ± 299	1150 ± 56.9 | 1820 ± 289	1150 ± 208 | 2350 ± 289	1110 ± 221 | 2020 ± 349
TDS (ppm)	828 ± 30.9 | 1460 ± 238	817 ± 36.8 | 1440 ± 219	815 ± 40.7 | 1100 ± 90.1	812 ± 148 | 1670 ± 206	780. ± 156 | 1600 ± 274
Salinity (ppm)	540. ± 21.0 | 1010 ± 175	534 ± 25.2 | 1000 ± 159	534 ± 28.1 | 948 ± 122	535 ± 101 | 1150 ± 142	550. ± 99.7 | 1110 ± 180.
TAN (mg/L)	0.463 ± 0.183 | 2.32 ± 1.65	0.893 ± 0.433 | 2.23 ± 1.53	1.20 ± 0.481 | 2.26 ± 1.42	0.120 ± 0.032 | 0.043 ± 0.019	0.040 ± 0.017 | 0.027 ± 0.017
TNN (mg/L)	11.3 ± 0.734 | 10.9 ± 0.508	9.54 ± 1.16 | 10.4 ± 0.717	9.14 ± 1.04 | 9.52 ± 1.16	2.10 ± 0.804 | 1.09 ± 0.135	1.66 ± 0.667 | 1.15 ± 0.063
TN (mg/L)	13.1 ± 1.03 | 16.1 ± 3.21	12.2 ± 1.41 | 15.1 ± 3.66	12.2 ± 1.76 | 14.6 ± 4.01	2.81 ± 0.868 | 1.50 ± 0.094	2.62 ± 0.959 | 1.54 ± 0.093
SRP (mg/L)	0.228 ± 0.048 | 0.187 ± 0.052	0.228 ± 0.055 | 0.187 ± 0.058	0.228 ± 0.058 | 0.193 ± 0.058	0.028 ± 0.016 | 0 ± 0	0 ± 0 | 0.023 ± 0.023
TP (mg/L)	0.452 ± 0.048 | 0.443 ± 0.038	0.406 ± 0.057 | 0.374 ± 0.052	0.361 ± 0.065 | 0.364 ± 0.065	0.13 ± 0.02 | 0.080 ± 0.011	0.157 ± 0.067 | 0.081 ± 0.023

two principal components revealed that sites closer to the outfall clustered together and were more distinct than sites upstream (Fig. 4C and D). Of all the water quality parameters, only water temperature was higher in the summer than in winter; whereas pH, conductivity, total dissolved solids, salinity, total ammonia nitrogen ($p = 0.09$) were all higher in the winter. In the summer, total nitrate nitrogen, total nitrogen, soluble reactive phosphorus, and total phosphorus decreased significantly with distance from the outfall, while the reverse trend was true for water temperature and pH. In the winter, water temperature, total ammonia nitrogen ($p = 0.08$), total nitrate nitrogen, total nitrogen, soluble reactive phosphorus, and total phosphorus decreased with distance from the outfall, whereas dissolved oxygen showed the opposite pattern (all contrasts were $p < 0.05$, unless otherwise stated). Similar to the Dundas WWTP, longer-term surface water temperatures were not significantly different between the outfall (W1) and reference (W4) sites during the summer (LM, $t_{(\text{Summer})} = 0.42$; $p = 0.68$; Fig. 5C), while in the winter, the outfall site was on average ~ 9 °C warmer than the reference site ($t_{(\text{Winter})} = 22.30$; $p < 0.001$; Fig. 5D). See Table 2 for further details on all water quality parameters.

All sites near the Woodward WWTP were anthropogenically disturbed with clear modifications to the shoreline, close proximity to urban structures, and relatively narrow riparian zones. Sites upstream (W4 and W5) however, appeared to be marginally less disturbed. While vegetation surrounding the water was different between seasons, all other habitat characteristics remained relatively unchanged between seasons. Further habitat characteristics are presented in Supplementary Table 7.

3.3. Effluent CECs characterization

3.3.1. Dundas WWTP

In the summer effluents, 16 out of the 21 compounds analyzed were detected at least once, while in the winter, all compounds were detected (Table 3). Overall, gemfibrozil, atorvastatin, p-hydroxy atorvastatin, o-hydroxy atorvastatin (lipid regulators and metabolites), carbamazepine (antiepileptic), and caffeine (stimulant) were detected at higher concentrations in the winter. Acetaminophen (analgesic) was only detected once during the winter and never in the summer. Antibacterials showed mixed patterns, where concentrations of triclosan were higher in the winter, while the opposite was true for sulfamethazine. Two of the four antibiotics analyzed (trimethoprim and sulfamethoxazole) were detected at higher concentrations in the winter. Monensin was detected only twice in the winter and not at all in the summer, whereas lincomycin was not detected in the winter and was only detected once in the summer. Fluoxetine, norfluoxetine, venlafaxine, and desvenlafaxine (antidepressants and metabolites) were all detected at higher concentrations in the winter. Two of the three nonsteroidal anti-inflammatory drugs analyzed (naproxen and diclofenac) were detected at higher concentrations in the winter, whereas ibuprofen concentrations were too variable to detect any significant differences between

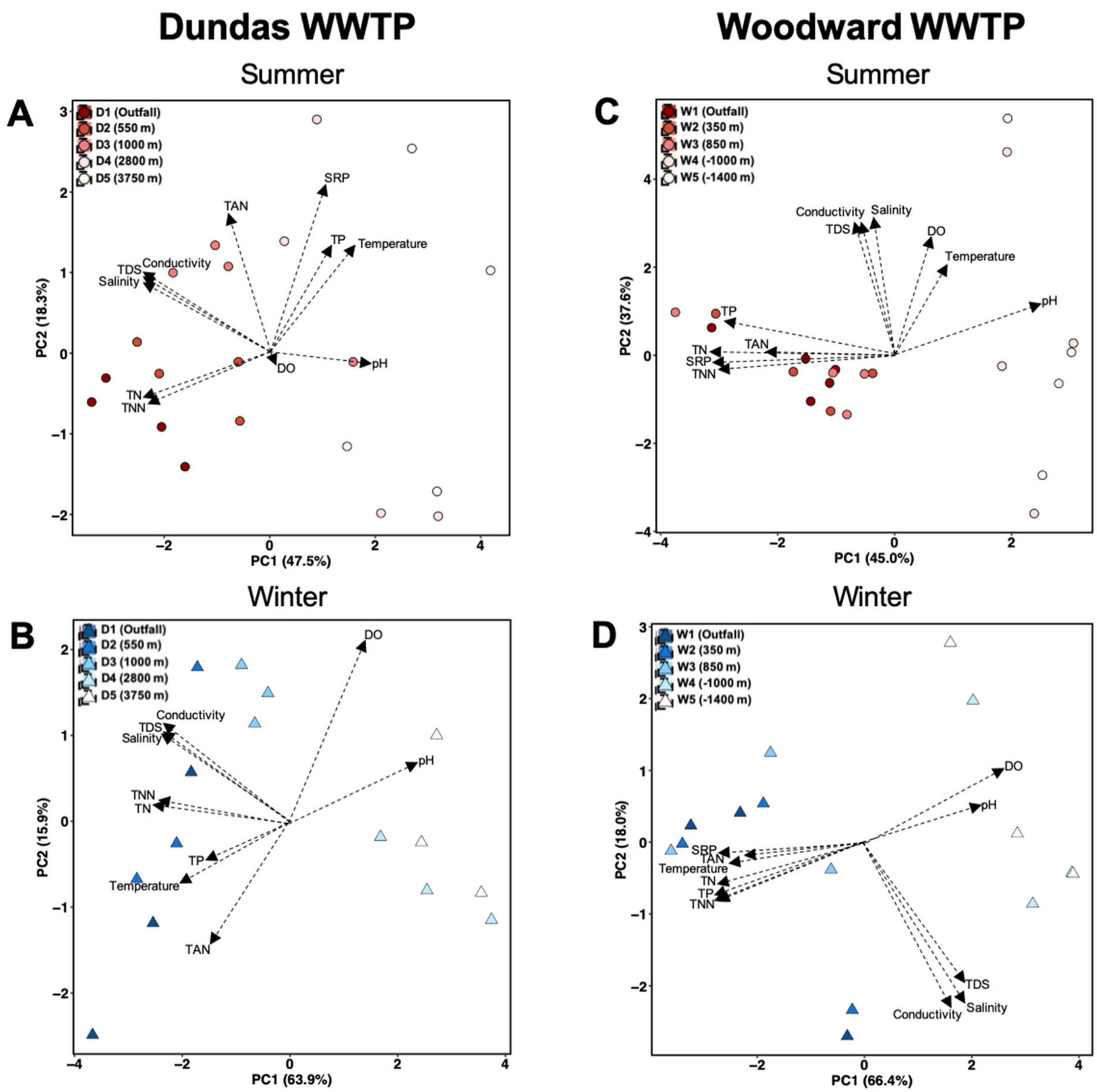

Fig. 4. Principal component analysis (PCA) biplots on water quality parameters measured along the effluent gradients of both WWTPs. (A, B) PCA biplots from the Dundas WWTP in summer and winter, respectively. (C, D) PCA biplots from the Woodward WWTP in summer and winter, respectively. Each data point represents a field sampling event. The dashed lines represent the strength of the loadings and direction of the respective water quality parameters in two-dimensional space. See Supplementary Table 11 for PCA loadings.

seasons. Finally, atrazine (herbicide) was detected at higher concentrations in the summer.

3.3.2. Woodward WWTP

The effluent characteristics from the Woodward WWTP displayed distinct seasonal patterns. While the same 20 out of the 21 compounds analyzed were detected in both seasons, the concentrations varied considerably between the summer and winter (Table 3). Three of the four lipid regulators and metabolites analyzed were detected at higher concentrations in the winter (atorvastatin, p-hydroxy atorvastatin, and o-hydroxy atorvastatin); however, gemfibrozil was not significantly different between seasons. Neither carbamazepine (antiepileptic) nor acetaminophen (analgesic) varied significantly between seasons, while caffeine (stimulant) was detected at higher concentrations in the winter. Similar to the effluent from the Dundas WWTP, concentrations of antibacterials showed mixed patterns. Triclosan was detected at higher concentrations in the winter, while sulfamethazine was higher in summer. Of the four antibiotics analyzed, only lincomycin was detected at higher concentrations in the summer, while concentrations of sulfamethoxazole and trimethoprim did not significantly differ between seasons. Monensin was not detected in either season. Both antidepressants, fluoxetine and venlafaxine, were detected at higher concentrations in the summer, however, the concentrations of both metabolites (norfluoxetine and desvenlafaxine) were higher in the winter. Two of the three non-steroidal anti-inflammatory drugs analyzed (ibuprofen and naproxen) were detected at higher concentrations in the winter, while diclofenac concentrations were not significantly different between seasons. Finally, atrazine (herbicide) was detected at higher concentrations in the summer.

4. Discussion

Our study explored the impacts of wastewater contamination on fish communities between the summer and winter. Fish communities were sampled along contamination gradients generated by two WWTPs in Hamilton, ON, Canada during both seasons. In the winter,

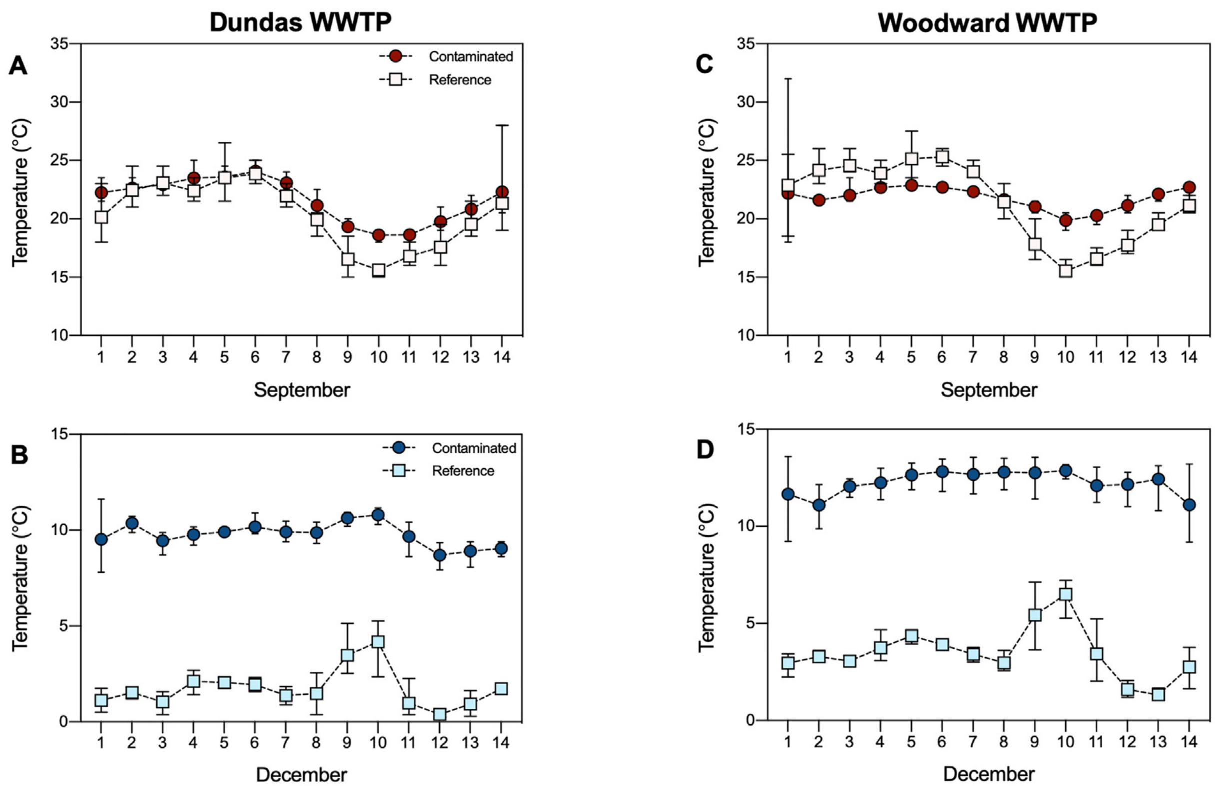

Fig. 5. Mean, minimum, and maximum daily temperatures of the outfall site (D1, W1) and one reference site (D4, W4) measured along a 14-day period in the summer and winter. (A, B) Temperature data from the Dundas WWTP in summer and winter, respectively. (C, D) Temperature data from the Woodward WWTP in summer and winter, respectively. Temperature data was recorded once every 30 min in the summer and once every 15 min in winter.

sites closer to the effluent outfall generally had higher fish abundance, higher species richness, and higher species diversity compared to sites farther away. This trend, however, was not as apparent in the summer. Wastewater plumes are a significant source of nutrients in aquatic environments, so fish may seek such highly productive environments, especially during the winter, when food is scarce and difficult to encounter (Sommer et al., 1986; Byström et al., 2006; Hurst, 2007; Holeton et al., 2011). The increase in productivity in effluent-receiving environments likely leads to higher food availability and is potentially the cause of the increase in growth and body condition observed in fishes caught in such environments (Chambers et al., 1997; McMaster et al., 2005; Brown et al., 2011; Tetreault et al., 2011). Additionally, effluent released from WWTPs can significantly alter the thermal conditions of receiving environments (Environment Canada, 2001; Kinouchi et al., 2007; Mehdi

Table 3
Mean (±SE) concentrations in [ng/L] of various classes of chemicals measured in the final effluent of the Dundas and Woodward WWTPs in summer ($n_{Dundas} = 7$, $n_{Woodward} = 8$) and winter ($n_{Dundas} = 8$, $n_{Woodward} = 8$). Zeros indicate concentrations measured below detection limit. Bolded averages indicate a significant difference ($p < 0.05$) between seasons within each WWTP.

		Dundas WWTP		Woodward WWTP	
		Summer	Winter	Summer	Winter
Lipid regulator	Gemfibrozil	0 ± 0	**9.65 ± 2.80**	13.2 ± 3.42	9.76 ± 1.18
	Atorvastatin	28.2 ± 0.672	**305 ± 20.9**	23.3 ± 0.867	**292 ± 17.1**
	p-Hydroxy atorvastatin	160. ± 37.8	**484 ± 45.2**	126 ± 13.1	**425 ± 20.5**
	o-Hydroxy atorvastatin	18.3 ± 5.41	**480 ± 38.4**	46.7 ± 5.84	**431 ± 19.3**
Antiepileptic	Carbamazepine	293 ± 7.59	**406 ± 23.4**	310 ± 14.1	268 ± 20.0
Analgesic	Acetaminophen	0 ± 0	2.56 ± 2.56	30.1 ± 30.1	16.4 ± 6.33
Stimulant	Caffeine	15.8 ± 0.889	**212 ± 122**	218 ± 43.4	**494 ± 70.1**
Antibacterial	Triclosan	0 ± 0	**22.4 ± 1.75**	36.9 ± 3.88	**62.2 ± 2.99**
	Sulfamethazine	**3.61 ± 2.63**	0 ± 0	**64.0 ± 17.8**	7.84 ± 2.71
Antibiotic	Monensin	0 ± 0	0.333 ± 0.221	0 ± 0	0 ± 0
	Trimethoprim	7.96 ± 4.46	**223 ± 14.0**	156 ± 17.0	136 ± 8.17
	Lincomycin	0.313 ± 0.313	0 ± 0	**10.6 ± 3.75**	1.14 ± 1.08
	Sulfamethoxazole	12.9 ± 2.63	**651 ± 51.8**	325 ± 32.8	398 ± 44.6
Antidepressant	Fluoxetine	14.1 ± 11.7	**45.5 ± 0.938**	**88.2 ± 11.6**	26.6 ± 0.502
	Norfluoxetine	5.30 ± 1.05	**9.67 ± 0.505**	3.31 ± 0.492	**9.48 ± 0.340**
	Venlafaxine	195 ± 65.8	**939 ± 68.0**	**865 ± 52.9**	578 ± 46.1
	Desvenlafaxine	6.13 ± 2.73	**1830 ± 110**	696 ± 70.6	**1060 ± 78.5**
NSAID	Ibuprofen	6.99 ± 1.82	231 ± 162	90.0 ± 14.9	**530 ± 184**
	Naproxen	0 ± 0	**252 ± 115**	141 ± 16.2	**583 ± 32.0**
	Diclofenac	176 ± 18.3	**1090 ± 41.8**	720 ± 48.0	802 ± 42.9
Herbicide	Atrazine	**32.9 ± 1.02**	25.2 ± 1.17	**36.2 ± 1.70**	25.6 ± 1.89

et al., 2019). In the summer, we observed that temperature increased with distance from the outfall, while during the winter, sites closer to the outfall were significantly warmer than sites farther away. Sites closer to the outfall also appeared to be more thermally stable than sites farther away. Thermal enhancement of effluent-receiving environments may create perceived thermal refugia for aquatic organisms, particularly during the winter, when survival elsewhere may be challenging (Brodersen et al., 2011). Fish may select sites with temperatures closer to their optima (i.e., sites closer to the outfall), rather than sites with cooler temperatures (i.e., sites farther away from the outfall; Cooke et al., 2000, 2004). The combination of a steady supply of nutrients and thermal enhancement in effluent-receiving environments may create an enticing ecological trap for aquatic organisms, as these environments may be perceived by individuals as beneficial or favourable, but are also a major source of contamination, and may pose detrimental costs to reproduction and survival (Schlaepfer et al., 2002; Battin, 2004; Holeton et al., 2011). Empirical assessment of fish thermal preferences and food availability across seasons in wastewater-impacted environments would be a natural next step for understanding why fish might be attracted to such environments.

During the summer, fish community composition (dissimilarity, tolerance, proportion of native to non-native species, and the average trophic level) differed along the effluent gradients at one or both WWTPs, with the outfall sites being most dissimilar from the reference sites. Previous studies conducted in the summer have also demonstrated that wastewater promotes the presence of tolerant, non-native, and omnivorous fishes (Tetreault et al., 2012; McCallum et al., 2019). A study conducted along the same effluent gradients as our own found that both fish abundance and species richness was highest near the outfalls during the summer (McCallum et al., 2019). Our study demonstrated a similar pattern in the sites closest to the outfalls, however, sites farther away were relatively more variable. Such differences may be attributable to our study using 1) more gear types, 2) different reference sites, and 3) sampling over a shorter period (July and August only). In our study, fish abundance, species richness, and species diversity, and overall composition varied between the two WWTPs. Such varying patterns between the two WWTPs indicate that it is difficult to generalize the impacts of wastewater contamination on fish communities. The different results may be due, in part, to WWTPs varying in almost every aspect (e.g., population served, daily capacity, treatment technologies; see Methods section for differences between the two WWTPs). Furthermore, the habitats that wastewater effluent is discharged into can also influence fish community responses (Midwood et al., 2015; McCallum et al., 2019; see Supplementary Tables 6 and 7). Despite such differences, key similarities were identified. Specifically, more fish were found near the outflows and certain key species were present in sites closer to the outfall (e.g., round goby, white sucker, green sunfish, and spottail shiner). Previous studies have demonstrated that these species are often found in polluted environments and tend to be tolerant and/or resilient to a wide range of environmental conditions (e.g., dissolved oxygen, temperature, salinity, turbidity, and pollution; Reash and Berra, 1987; Froese and Pauly, 2020; Eakins, 2018; Anseeuw et al., 2012; Kornis et al., 2012; Hernandez, 2014). The overrepresentation of these species in impacted sites may indicate that only certain species are able to tolerate wastewater exposure while reaping the benefits associated with polluted environments. It is important to note that during the winter, species richness and species diversity were both significantly higher in sites closer to the outfall, especially at the Dundas WWTP, suggesting fishes are either staying and/or moving into wastewater plumes. Telemetry studies tracking movement of fishes in and out wastewater plumes across seasons would be critical in further understanding how wastewater outfalls may act as ecological traps.

Water quality parameters varied along the effluent gradients generated by both WWTPs and between seasons, further establishing the impacts that municipal wastewater can have on the chemical and physical characteristics of effluent-impacted environments. Additionally, of the chemical compounds analyzed, the majority were more frequently detected and detected at higher concentrations in the winter compared to summer, implying that contaminant loading worsens during the winter. Similar trends have previously been demonstrated in other countries that experience comparable climates to Canada, e.g., China (Sui et al., 2011), Finland (Vieno et al., 2005), Poland (Kot-Wasik et al., 2016), and the USA (Yu et al., 2013). Such seasonal differences in the concentrations of PPCPs have previously been attributed to increased pharmaceutical consumption and to poorer biological degradation of contaminants in WWTPs during colder months of the year. Indeed, antibiotics, analgesics, and antidepressants are more likely to be prescribed during the winter (Vieno et al., 2005; Gardarsdottir et al., 2010; ter Laak et al., 2010; Sui et al., 2011; Yu et al., 2013; Suda et al., 2014); however, we could not find data on seasonal pharmaceutical prescription and consumption rates of the specific compounds we measured in Canadian populations. Seasonal differences in the sewershed flows and inputs as well as in treatment efficiencies may have influenced the CECs distribution in the final effluent. In the Dundas WWTP effluent, but not in the Woodward WWTP, there was a distinct increase in treatment-resistant compounds such as carbamazepine and venlafaxine during the winter. Further studies are needed to better understand the seasonal distribution of these chemicals entering the environment. Several of the compounds analyzed in our study have been linked to sublethal effects in fishes (e.g., venlafaxine and fluoxetine), including impacts on metabolism (Best et al., 2014; Mennigen et al., 2010; Mehdi et al., 2019), stress response (Ings et al., 2011a, 2011b; Melnyk-Lamont et al., 2014), reproductive capacity (Lister et al., 2009; Weinberger and Klaper, 2014), and routine behaviours (Martin et al., 2017; McCallum et al., 2017b; Martin et al., 2019). However, these impacts may be more severe in the winter, as the metabolic scope of ectotherms is greatly reduced in colder temperatures, potentially limiting the capacity to detoxify contaminants found in wastewater effluent (Lemly, 1993; Lemly, 1996). Moreover, fish may also suffer from endogenous exposure to contaminants during the winter as reliance on lipid stores expectedly increases, which may mobilise tissue stores of some contaminants (Paterson et al., 2007; Treberg et al., 2016). Also, lower water temperatures in the winter may reduce metabolism and gill ventilation, thereby decreasing uptake, elimination, and remobilization of contaminants (Capkin et al., 2006; Buckman et al., 2007; Noyes et al., 2009). The physiological impacts of winter temperatures on fish contaminant exposures downstream of WWTP outfalls warrant further exploration.

Few compounds were present in effluents at higher concentrations in the summer. Atrazine, lincomycin, monensin, and sulfamethazine are predominantly used in agriculture, making their seasonal concentrations less surprising (Couperus et al., 2016). While most of the compounds assessed in our study were measured at concentrations lower than what would be considered lethal (Brausch and Rand, 2011; Brausch et al., 2012), it should be noted that there are no water quality guidelines for the majority of PPCPs found in wastewater effluents. However, ammonia is a pollutant that is actively monitored in wastewater treatment facilities, as it is toxic in freshwater ecosystems (Canadian Council of Ministers of the Environment, 2010). Mean LC_{50} values reported for freshwater fishes typically range from 0.56 to 2.37 mg/L, where toxicity is often higher at lower temperatures and pH values (Environment Canada, 1999). These concentrations are within the range of what we observed in our study, especially downstream of the Woodward WWTP during the winter (up to 5.62 mg/L ammonia/ammonium). Ammonia exposure has been linked to numerous adverse effects in fishes, including reproductive and developmental impairments as well as morphological abnormalities (Randall and Tsui, 2002; Yuen and Chew, 2010). High concentrations of toxic nitrogenous products, like those found in wastewater effluent, may significantly disturb fish populations and communities in effluent-receiving environments, especially in the presence of other contaminants (Environment Canada, 1999; Canadian Council of Ministers of the Environment, 2010; EPA, 2013). Hence, our study further emphasizes the importance of winter research in ecotoxicology, as research conducted only in warmer seasons may not reveal the full scope of the impacts of wastewater effluent in aquatic ecosystems.

This study is unique in its approach to studying the impacts of wastewater on fish communities and water quality during the winter—a season seldom studied in ecotoxicology. We demonstrated that effluent-receiving environments may act as ecological traps for fishes, especially during the winter, where the effluent provides enhancement and stability to the temperature profile of receiving water bodies, as well as potentially increasing the availability of food when it is scarce elsewhere. Effluent quality was also predictably worse during the winter compared to the summer. To better understand why fish might choose sites with greater contaminant exposure, future research should further investigate the relative costs and benefits of living in effluent-dominated environments using lab and field manipulation studies in fish and other aquatic organisms, especially during the winter. Such research would aid conservation and management efforts of aquatic ecosystems that are heavily impacted by wastewater pollution and urbanization.

CRediT authorship contribution statement

Hossein Mehdi: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Samantha C. Lau:** Investigation, Writing - review & editing. **Caitlyn Synyshyn:** Investigation, Writing - review & editing. **Matthew G. Salena:** Investigation, Writing - review & editing. **Erin S. McCallum:** Investigation, Formal analysis, Writing - review & editing. **Melissa N. Muzzatti:** Investigation, Writing - review & editing. **Jennifer E. Bowman:** Investigation. **Kyle Mataya:** Investigation. **Leslie M. Bragg:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing. **Mark R. Servos:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Karen A. Kidd:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Graham R. Scott:** 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143430.

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