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Municipal wastewater effluent affects fish communities: A multi-year study involving two wastewater treatment plants☆

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ABSTRACT

Although effluent from municipal wastewater treatment plants (WWTPs) is a major stressor in receiving environments, relatively few studies have addressed how its discharge affects natural fish communities. Here, we assessed fish community composition over three years along a gradient of effluent exposure from two distinct WWTPs within an International Joint Commission Area of Concern on the Great Lakes (Hamilton Harbour, Canada). We found that fish communities changed with distance from both WWTPs, and were highly dissimilar between sites that were closest to and furthest from the wastewater outfall. Despite differences in the size and treatment technology of the WWTPs and receiving habitats downstream, we found that the sites nearest the outfalls had the highest fish abundances and contained a common set of signature fish species (i.e., round goby *Neogobius melanostomus*, green sunfish *Lepomis cyanellus*). Non-native and stress tolerant species were also more abundant near one of the studied WWTPs when compared to the reference site, and the number of young-of-the-year fish collected did not vary along the effluent exposure gradients. Overall, we show that fish are attracted to wastewater outfalls raising the possibility that these sites may act as an ecological trap.

1. Introduction

Freshwater environments near urban centres often face a complex mix of anthropogenic stressors that can adversely alter environmental conditions (e.g., eutrophication, habitat modification, urban-run off, and pollutants) (Dudgeon et al., 2006; Reid et al., 2018; Vörösmarty et al., 2010). Effluents from wastewater treatment plants (WWTP) are a common stressor in urban areas, contributing excess nutrients (e.g., nitrogen, phosphorus) and reducing dissolved oxygen that can lead to eutrophication (Carey and Migliaccio, 2009; Jarvie et al., 2006). Wastewater effluent is also a significant source of emerging and legacy pollutants such as metals, pesticides, detergents, plastic by-products, and pharmaceuticals and personal care products (PPCPs) (Kolpin et al., 2002; Loos et al., 2013). WWTPs are not specifically designed to degrade such contaminants and many are poorly or incompletely removed during typical wastewater treatment processes (Jelić et al., 2012; Luo et al., 2014; Verlicchi et al., 2012). As human use of synthetic products rises and urban populations grow, the impacts of wastewater effluent on aquatic organisms is of increasing concern (Bernhardt et al., 2017; Chambers et al., 1997; Sumpter, 2009).

Wild fish collected downstream from WWTP outfalls show signs of biological disruption, spanning molecular to whole-organism responses, including altered gene expression (Bahamonde et al., 2014, 2015), external male feminization and gonadal intersex (Tetreault et al., 2011; Tyler and Jobling, 2008; van Aerle et al.,

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2001), poor fertilization success (Fuzzen et al., 2015; Harris et al., 2012), increased metabolism (Du et al., 2018; Mehdi et al., 2018), impaired stress responsiveness (Pottinger et al., 2013), and abnormal courtship and aggressive behaviours (McCallum et al., 2017b; Saaristo et al., 2014; Simmons et al., 2017). These effects were often linked to the presence of WWTP contaminants (e.g., synthetic and natural estrogens) in the environment from which the fish were collected. However, not all studies have found that wastewater effluent exposure affects fish physiology and/or behaviour, suggesting that some species are more tolerant than others (Douxfils et al., 2007; Körner et al., 2007; McCallum et al., 2017a; Schoenfuss et al., 2002).

In contrast to studies on how WWTP effluents affect individual fish, comparatively few have addressed how fish communities respond to wastewater effluent. This is surprising because fish community structure is commonly used as an indicator of water and habitat quality, especially in environments impacted by human activities (Cvetkovic et al., 2010; Fausch et al., 1990; Karr, 1981). Of the studies conducted to date on wastewater effluent, fish communities near wastewater outfalls were found to be less species rich and comprised of more tolerant species and/or omnivorous species (Dyer and Wang, 2002; Northington and Hershey, 2006; Ra et al., 2007; Tetreault et al., 2013). However, most studies have assessed community responses over short timeframes whereas monitoring over longer periods would help identify common responses to wastewater effluent that may be broadly applicable across water bodies. Such studies would also benefit from the use of several metrics to describe aquatic communities (catch per unit effort, species richness, proportional abundance of young-of-the-year and invasive/tolerant species) in order to provide a comprehensive evaluation of species composition and community quality.

In this study, we evaluated spatial changes in fish community composition near two WWTPs across multiple years in an International Joint Commission Area of Concern on the Great Lakes (Hamilton Harbour, Canada; Great Lakes Water Quality Agreement, 2012; Hamilton Harbour Remedial Action Plan, 1992; International Joint Commission, 1999). We also related changes in fish community composition to changes in water quality and habitat characteristics across sampling sites. Located at the western-most edge of Lake Ontario, Hamilton Harbour and adjoining Cootes Paradise Marsh are undergoing remediation to improve water quality, aquatic habitats, and fish and wildlife populations that were severely degraded by the combination of urbanization, industrial activities, habitat modification, invasive species, and wastewater effluent inputs since the late 1800's (Hamilton Harbour Remedial Action Plan, 1992; Hall and Connor, 2016; Thomasen and Chow-Fraser, 2012). Wastewater effluent is a large stressor in this ecosystem, as approximately 50% of the water flow into Hamilton Harbour is from WWTPs (Government of Canada, 2017). Emerging pollutants such as PPCPs that are commonly associated with WWTPs have recently been measured in water and fish from Hamilton Harbour and Cootes Paradise Marsh (Csiszar et al., 2011; McCallum et al., 2017a, 2017b; Muir et al., 2017). Additionally, Cootes Paradise Marsh is a nature sanctuary that is a vital stopover site for migratory waterfowl, and a significant source of wetland reptile-, amphibian- and fish-spawning habitat for western Lake Ontario (Leslie and Timmins, 1992; Smith and Chow-Fraser, 2010). We predicted that there would be fewer fish species, more non-native and/or stress tolerant fishes, and fewer YOY fish at sites near the wastewater outfalls (Brown et al., 2011; Porter and Janz, 2003; Ra et al., 2007; Tetreault et al., 2013). However, stable effluent temperatures and nutrients can also attract fish to outfalls (Azzurro et al., 2010; Hall et al., 1997; Smith and Bailey, 1990).

2. Methods

2.1. Sampling regime and study areas

2.1.1. Sampling regime

Our fish community sampling began in 2016 at the Dundas WWTP (described further in section 2.1.2) and expanded in 2017 to include the Woodward WWTP (described further in section 2.1.3). In 2016, we sampled 4 sites downstream from the Dundas WWTP and in 2017 and 2018, we added an additional reference site (5 sites in total) along the Dundas WWTP effluent gradient (Fig. 1). In 2017, 2018, we sampled 4 sites along the Woodward WWTP effluent gradient (Fig. 1). We sampled all sites between June and October on either 2 (2018) or 3 (2016 and 2017) occasions per year (see below for more details).

2.1.2. Dundas WWTP

The Dundas WWTP is a conventional activated sludge treatment facility with nitrification and tertiary sand filtration (City of Hamilton, 2019). The facility serves the population of Dundas, near Hamilton, ON (~30 000 people) and treats a daily average of 14.6 million litres of wastewater (City of Hamilton, 2019). The effluent is discharged into the western-most end of the Desjardins Canal, the remnants of a dredged shipping corridor that once connected Dundas to Hamilton Harbour (Theysmeyer and Bowman, 2017). Wastewater effluent is the main source of water flow into the Desjardins Canal, with no other inputs except for a small contribution from a run-off ditch (Hamilton Water, unpublished data). Our sampling sites (see Fig. 1)—ordered from most to least impacted by wastewater effluent—included an Outfall site, which was immediately adjacent to the WWTP outfall; Downstream 1, which was 550 m downstream; Downstream 2, which was 1000 m downstream and receives additional seasonal flow inputs from Delsey Creek. Two references sites were also examined and neither was located directly in the flow of the wastewater effluent: Reference 1 was located 2080 m from the outfall at the southwestern edge of Cootes Paradise Marsh; Reference 2, which was located in the mouth of Spencer Creek, 2800 m from the outfall. The second reference site was added to our sampling regime in 2017 because the water flow and habitat structure at this site better resembled that of our wastewater-exposed sites compared to reference 1. We sampled these sites at eight times over three years on June 21, August 18, and September 22 in 2016, June 20, August 15, and October 13 in 2017, and July 7 and August 14 in 2018.

2.1.3. Woodward WWTP

The Woodward WWTP is a secondary conventional activated sludge plant that serves most of the population of urban Hamilton, including Stoney Creek and Ancaster (~480 000 population, City of Hamilton, 2019). This facility handles wastewater from a combined (40%) and separated (60%) sewer system, and treats a daily average of 409 million litres of wastewater (City of Hamilton, 2019). Effluent from the facility flows into Hamilton Harbour *via* the Red Hill Creek (43°15′44.14″N, 79°46′20.71″W), bypassing the Windermere Basin. Our sampling sites (Fig. 1)—ordered from most to least impacted by wastewater effluent—included Outfall, which was adjacent to the WWTP outfall; Downstream 1, which was 350 m downstream from the outfall; Downstream 2, which was located 850 m downstream, immediately before the inlet to the Windermere Basin; and Reference, which was located in Red Hill Creek, 800 m upstream of the WWTP outfall. We sampled all four sites along the wastewater gradient at five times over two years on June 22, August 11, and October 11 in 2017, and July 17 and August 22 in 2018.

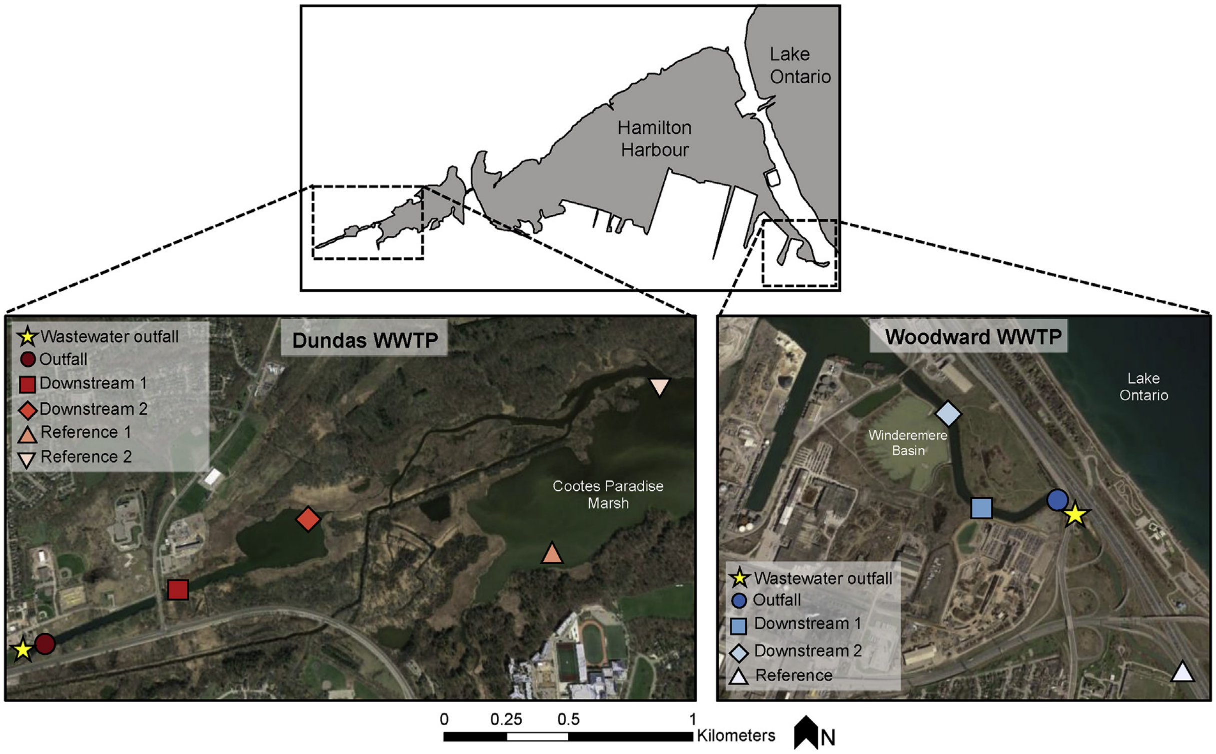

Fig. 1. Map of Hamilton Harbour, ON with two insets depicting sampling sites downstream from the Dundas and Woodward WWTPs (Google Earth Pro, version 7.3.1.4507. Imagery taken on April 14, 2017, Terra Metrics and accessed on April 12, 2018).

2.2. Fish sampling

We measured fish communities using two sampling techniques: passive sampling gear (minnow traps) and active sampling gear (electrofishing from a boat). Seven to ten minnow traps baited with corn (~20 g per minnow trap) were set ~10 m apart at each site and retrieved after 24 h. We also electrofished one transect at each site (50 m in 2016, 100 m in 2017 and 2018; 1.5-KVA Electrofisher, Smith-Root Inc.). We identified all fish species caught with both gear types, and measured fork length or standard length (depending on the species) to estimate developmental stage based on body size (YOY or adult, and later confirmed with reference measures from Scott and Crossman, 1998) for up to 15 individuals of each species and each developmental stage captured. After those targets were reached, we counted the remaining fish by species and developmental stage. Tolerance was categorized following Eakins (2018) and describes the species' ability to adapt to environmental perturbations or anthropogenic stressors (see Table 1). Resilience was categorized following Froese and Pauly (2019), and describes the species ability to recover after exploitation based on their estimated population doubling time (low > 4 years, medium 1.4–4 years, high < 1.4 years; Table 1).

2.3. Water quality and habitat characterization

To assess water quality, we measured several parameters at each sampling site and on each sampling date. Water quality meters were always calibrated before use in the field and were used to measure dissolved oxygen (DO), temperature (both with Extect Pocket Tracer 2016/17 or YSI ProODO in 2018), pH, conductivity (Cond), total dissolved solids (TDS; all using Oakton multi parameter Testr), flow (m/s using a Höntzsch wheel flow meter), and water clarity (Secchi disk). Additionally, we collected water samples for nutrient analysis using a 2.2 L Van Dorn sampler deployed at mid-depth and dispensed the sample into clean plastic bottles (Corning brand, acid washed and rinsed with de-ionized water). In 2016, all samples were analyzed following the methods outlined in Chow-Fraser (2006). Water samples for total ammonia nitrogen (TAN) were analyzed within 24 h of collection. Water samples for total nitrogen (TN), total nitrate nitrogen (TNN), total phosphorus (TP), and soluble reactive phosphorus (SRP) were frozen and later analyzed. We analyzed all samples for TAN, TNN, and TN using protocols and reagents from Hach (Hach Company www.hach.com/wah) and with a Hach DR2000 spectrophotometer (Hach, Loveland, Colorado, U.S.A.). Samples for TP were digested in potassium persulfate and measured using the molybdenum blue method of Murphy and Riley (1962). Samples for soluble reactive phosphorus (SRP) were passed through 0.45 μm filters before measurement with molybdenum blue, without digestion. In 2017, 2018, all samples were analyzed at the City of Hamilton Environmental Laboratory. TAN was analyzed using a San++ Continuous Flow Analyzer (Skalar). TNN was measured using Anion Chromatography, and TN, TP and SRP were analyzed using Colourimetric methodology (see Supplementary Materials for more details). Water quality findings are summarized in Table 2.

We characterized the habitat at each site for a subset of the metrics following the methods used in the Qualitative Habitat Evaluation Index (QHEI) for Lake/Lacustuary habitats (see Strickland et al., 2010; Taft and Koncelik, 2006). Briefly, we measured substrate type by collecting three samples of sediment every 10 m along each of the transects where fish were collected. We characterized the substrate on site when it was too large to be collected (i.e., cobble, boulders). We measured sediment particles using a dissecting microscope and then classified them based on QHEI size criteria. We estimated the dominant types of substrate along the transect (percentage, based on measured samples and

Table 1
Fish species characteristics and abundance from all collection years along a gradient of effluent exposure at the Dundas and Woodward Wastewater Treatment Plants (WWTPs). Tolerance describes a species ability to adapt to environmental perturbations or anthropogenic stressors (following Eakins, 2018). Resilience describes a species ability to recover after exploitation and captures the population doubling time (low > 4 years, medium 1.4–4 years, high, high < 1.4 years, Froese and Pauly, 2019). Abundance data are shown as the number caught by electrofishing and the number caught by minnow trapping (electrofishing/minnow trapping). – indicates no fish were caught with either method.

	Characteristics			Dundas WWTP					Woodward WWTP			
	Non-native/ Native	Tolerance	Resilience	Outfall	Down-stream 1	Down-stream 2	Reference 1	Reference 2	Outfall	Down-stream 1	Down-stream 2	Reference
Blacknose shiner[a]	Native	Intolerant	High	–	–	–	–	–	2/0	–	–	–
Black crappie[b]	Native	Tolerant	Medium	–	19/0	–	0/1	1/0	–	–	–	–
Bluegill sunfish[c]	Native	Intermediate	Medium	1/0	267/7	4/5	149/14	21/5	–	–	–	73/11
Bluntnose minnow[d]	Native	Intermediate	Medium	19/0	–	8/0	–	–	10/0	34/0	6/0	1/0
Bowfin[e]	Native	Intermediate	Low	1/0	–	–	1/0	–	–	–	–	1/0
Brook silverside[f]	Native	Intermediate	High	–	–	–	1/0	–	–	–	–	–
Brook stickleback[g]	Native	Intermediate	High	–	–	–	–	–	1/4	0/1	0/1	–
Brown bullhead[h]	Native	Intermediate	Medium	3/0	8/3	10/7	131/18	7/0	–	–	–	–
Central mudminnow[i]	Native	Tolerant	Medium	–	1/0	–	–	–	–	–	–	–
Common carp[j]	Non-native	Tolerant	Medium	2/0	1/0	–	3/0	1/0	2/0	–	–	2/0
Common shiner[k]	Native	Intermediate	Medium	–	3/0	1/0	–	–	–	–	–	4/0
Creek chub[l]	Native	Intermediate	Medium	–	–	–	–	–	–	5/0	–	–
Emerald shiner[m]	Native	Intermediate	High	–	–	–	–	–	–	–	–	8/0
Fathead minnow[n]	Native	Tolerant	High	4/1	–	–	1/0	–	94/1	23/0	57/0	162/1
Gizzard shad[o]	Non-native	Tolerant	Medium	2/0	3/2	105/1	8/0	1/0	–	–	–	1/0
Goldfish[p]	Non-native	Tolerant	Medium	17/0	40/0	41/3	210/7	0/0	2/0	1/0	3/5	11/0
Green sunfish[q]	Native	Tolerant	Medium	101/14	50/18	4/10	0/3	1/0	111/1	7/19	38/0	53/0
Largemouth bass[r]	Native	Tolerant	Low	22/0	54/1	6/1	8/1	5/0	4/0	–	1/0	4/0
Logperch[s]	Native	Intolerant	Medium	1/0	1/0	–	1/0	1/0	0/5	–	–	–
Longnose gar[t]	Native	Tolerant	Low	–	–	–	–	–	–	–	–	1/0
Northern pike[u]	Native	Intermediate	Low	–	2/0	–	–	–	–	–	–	1/0
Pumpkinseed[v]	Native	Intermediate	Medium	13/1	77/22	5/22	26/2	1/0	1/4	2/0	–	26/0
Round goby[w]	Non-native	Intermediate	Medium	35/194	18/27	–	0/1	0/5	6/55	9/1	–	6/5
Rudd[x]	Non-native	Tolerant	Low	–	0/8	0/1	–	2/0	–	–	–	–
Smallmouth bass[y]	Native	Intermediate	Medium	1/0	0/1	–	–	–	–	–	–	–
Spottail shiner[z]	Native	Intermediate	Medium	8/0	7/2	–	–	21/0	0/3	–	4/0	2/0
White crappie[aa]	Native	Tolerant	Medium	–	1/0	–	–	–	–	–	–	–
White perch[ab]	Non-native	Intermediate	Low	–	1/0	1/0	2/33	–	–	–	1/0	1/1
White sucker[ac]	Native	Tolerant	Low	24/1	8/0	10/0	–	4/0	51/1	37/0	6/3	1/0
Yellow perch[ad]	Native	Intermediate	Medium	4/6	90/34	9/24	15/5	42/6	0/1	–	1/0	4/0

[a] Notropis heterolepis.
[b] Pomoxis nigromaculatus.
[c] Lepomis macrochirus.
[d] Pimephales notatus.
[e] Amia calva.
[f] Labidesthes sicculus.
[g] Culaea inconstans.
[h] Ameiurus nebulosus.
[i] Umbra limi.
[j] Cyprinus carpio.
[k] Luxilus cornutus.
[l] Semotilus atromaculatus.
[m] Notropis atherinoides.
[n] Pimephales promelas.
[o] Dorosoma cepedianum.
[p] Carassius auratus.
[q] Lepomis cyanellus.
[r] Micropterus salmoides.
[s] Percina caprodes.
[t] Lepisosteus osseus.
[u] Esox Lucius.
[v] Lepomis gibbosus.
[w] Neogobius melanostomus.
[x] Scardinius erythrophthalmus.
[y] Micropterus dolomieu.
[z] Notropis hudsonius.
[aa] *Pomoxis annularis*.
[ab] Morone Americana.
[ac] Catostomus commersonii.
[ad] *Perca flavescens*.

Table 2
Mean ± standard deviation and (range) of water quality measures taken at each sampling site across all years (Dundas: 2016–2018; Woodward: 2017–2018). We measured total nitrogen (TN), total ammonia nitrogen (TAN), total nitrate nitrogen (TNN), soluble reactive phosphorus (SRP), total phosphorus (TP), dissolved oxygen (DO), total dissolved solids (TDS), and conductivity (Cond). See Methods section 2.3 and SI file for further details on sample collection and analyses.

	Dundas WWTP					Woodward WWTP			
	Outfall	Downstream 1	Downstream 2	Reference 1	Reference 2	Outfall	Downstream 1	Downstream 2	Reference
TN, mg/l	24.5 ± 8.9 (16.7–35.5)	20.6 ± 7.1 (14.2–35.7)	10.0 ± 3.7 (3.8–15.4)	1.7 ± 0.7 (0.8–3.0)	1.9 ± 0.6 (1.4–2.7)	17.2 ± 3.8 (12.6–22.5)	16.4 ± 3.4 (11.9–19.9)	14.2 ± 2.0 (11.4–16.7)	2.4 ± 1.0 (1.4–3.9)
TAN, mg/l	0.07 ± 0.04 (0.02–0.11)	0.10 ± 0.08 (0.02–0.27)	0.09 ± 0.09 (0.02–0.30)	0.02 ± 0.01 (0.01–0.05)	0.10 ± 0.04 (0.06–0.17)	0.79 ± 0.18 (0.61–1.08)	1.28 ± 0.68 (0.42–2.00)	1.60 ± 0.56 (0.79–2.16)	0.13 ± 0.06 (0.08–0.23)
TNN, mg/l	10.92 ± 8.15 (1.06–17.3)	8.55 ± 6.71 (1.08–17.6)	3.73 ± 3.12 (0.57–7.98)	0.06 ± 0.05 (0.01–0.10)	0.68 ± 0.37 (0.39–1.31)	9.38 ± 2.25 (6.87–12.6)	8.11 ± 2.36 (6.19–11.9)	6.07 ± 3.76 (0.70–11.3)	1.04 ± 0.90 (0.27–2.6)
SRP, mg/l	0.06 ± 0.05 (0.01–0.17)	0.04 ± 0.02 (0.01–0.07)	0.03 ± 0.02 (0.01–0.06)	0.06 ± 0.04 (0.01–0.32)	0.05 ± 0.02 (0.05–0.14)	0.24 ± 0.12 (0.11–0.37)	0.25 ± 0.10 (0.16–0.39)	0.25 ± 0.10 (0.17–0.40)	0.25 ± 0.01 (0.05–0.08)
TP, mg/l	0.13 ± 0.04 (0.08–0.23)	0.12 ± 0.03 (0.08–0.19)	0.19 ± 0.06 (0.13–0.33)	0.23 ± 0.07 (0.14–0.32)	0.11 ± 0.02 (0.08–0.14)	0.44 ± 0.15 (0.21–0.56)	0.41 ± 0.12 (0.28–0.53)	0.38 ± 0.12 (0.26–0.40)	0.13 ± 0.05 (0.09–0.20)
Temp, °C	20.73 ± 1.88 (17.1–22.6)	22.55 ± 2.89 (15.9–24.9)	23.10 ± 4.37 (13.1–27.4)	24.10 ± 5.22 (12.8–29.0)	20.96 ± 5.14 (13.0–26.6)	20.50 ± 0.96 (19.3–21.8)	20.70 ± 1.02 (19.3–22.1)	20.78 ± 1.13 (18.9–21.8)	21.82 ± 3.05 (17.2–25.6)
DO, mg/l	8.72 ± 3.06 (3.53–13.96)	10.25 ± 2.61 (6.17–13.71)	8.77 ± 3.27 (5.12–13.84)	8.64 ± 4.96 (2.81–18.78)	7.20 ± 2.46 (4.63–10.90)	5.50 ± 0.46 (4.95–6.23)	4.75 ± 1.12 (2.87–5.61)	3.64 ± 0.67 (2.52–4.14)	7.03 ± 3.45 (3.53–12.54)
pH	7.72 ± 0.35 (7.23–8.15)	7.95 ± 0.37 (7.42–8.52)	8.30 ± 0.70 (7.48–9.31)	8.43 ± 0.60 (7.66–9.48)	8.22 ± 0.31 (7.94–8.70)	7.12 ± 0.25 (6.91–7.54)	7.16 ± 0.15 (6.96–7.38)	7.24 ± 0.32 (6.92–7.77)	7.88 ± 0.25 (7.48–8.11)
TDS, ppm	776 ± 83 (668–895)	772 ± 84 (665–920)	780 ± 85 (652–944)	651 ± 70 (563–770)	646 ± 103 (569–821)	726 ± 185 (462–950)	757 ± 138 (628–972)	724 ± 166 (546–965)	713 ± 243 (504–1100)
Cond, µS	1094 ± 117 (947–1262)	1089 ± 120 (934–1298)	1085 ± 127 (927–1329)	889 ± 94 (794–1074)	911 ± 142 (801–1151)	1018 ± 265 (643–1339)	1065 ± 197 (885–1372)	1025 ± 166 (799–1356)	1010 ± 333 (716–1543)
Secch, cm	88 ± 42 (30–150)	59 ± 11 (40–75)	41 ± 21 (15–85)	30 ± 17 (10–70)	35 ± 17 (20–65)	89 ± 37 (28–120)	114 ± 44 (70–180)	89 ± 38 (48–130)	45 ± 26 (26–90)

visual inspection). In addition, we determined shoreline morphology (the slope from shore to bottom where the categories were based on angle degree; the degree of sinuosity where the categories were based on number of bends; and anthropogenic modifications which were reported from visual inspection), and measured riparian zone width (width of natural vegetation on the adjacent land), estimated percentage bank erosion, and we noted any species of aquatic plants present. All results of the habitat assessments are summarized in Supplementary Tables S1 and S2.

2.4. Statistical analyses

All statistical analyses were conducted using R (version: 3.5.1; R Core Team, 2018). We mean-standardized the fish sampling data from the minnow traps and electrofishing to allow them to be analyzed together (multi-gear mean standardization; Gibson-Reinemer et al., 2017; the "per unit effort" for electrofishing data was per shocking second and per trap deployed for minnow traps). We tested if abundance, species richness, and proportion of the catch comprised of non-native fish, YOY, tolerant fish, or resilient fish (high resilience) varied with increasing effluent exposure from the WWTPs using linear models, with year as a categorical factor and sampling site as an ordered factor. We tested for both linear and quadratic effects of sampling sites. When necessary, data were log transformed to meet model assumptions. To more reliably test our data given the small number of sampling opportunities, we used permutation tests of the same models to extract accurate p-values using 10,000 random permutations of the data. We used a multivariate principal coordinate analysis on all species collected (PCoA, also referred to as classic metric multidimensional scaling) with a Bray-Curtis dissimilarity matrix to assess how the fish community (fish abundances for each species) varied across our sampling sites at each WWTP (Borcard et al., 2011; Legendre and Legendre, 2003). We tested for sampling site and year differences using a permutation ANOVA following 10,000 permutations of the data (Borcard et al., 2011) adonis, vegan package: Oksanen et al. (2019). This analysis was followed with a similarity percentages analysis (SIMPER) to identify species driving the differences among sites (Oksanen et al., 2019). Only species that contributed to >80% of the dissimilarity between sites were further interpreted. We used a non-parametric Kruskal–Wallis (KW) test (with post-hoc when if necessary, PMCMR package, Pohlert, 2014) to determine whether there were significant differences in abundance among sites for these species (Midwood et al., 2015). Water quality measures (water chemistry and nutrients) were plotted and analyzed using a principal component analysis (PCA) using a standard Euclidean distance matrix (See Supplementary Table S3 for PCA loadings). We then tested for year and sampling site differences in water quality measures using a permutation ANOVA following 10,000 permutations of the data (Borcard et al., 2011; adonis, vegan package: Oksanen et al., 2019).

2.5. Ethical note

All fish handling and collection methods were approved by the McMaster University Animal Research Ethics Board (Animal Utilization Protocols: 13-12-51, 18-12-45). Following capture and measurement, all native fish species were released at their site of collection, and as required (Ontario Ministry of Natural Resources, 2015), all non-native fish species collected were euthanized with an overdose of benzocaine (0.025%, Sigma Aldrich) or by cerebral concussion if they were too large to fit in the chemical bath.

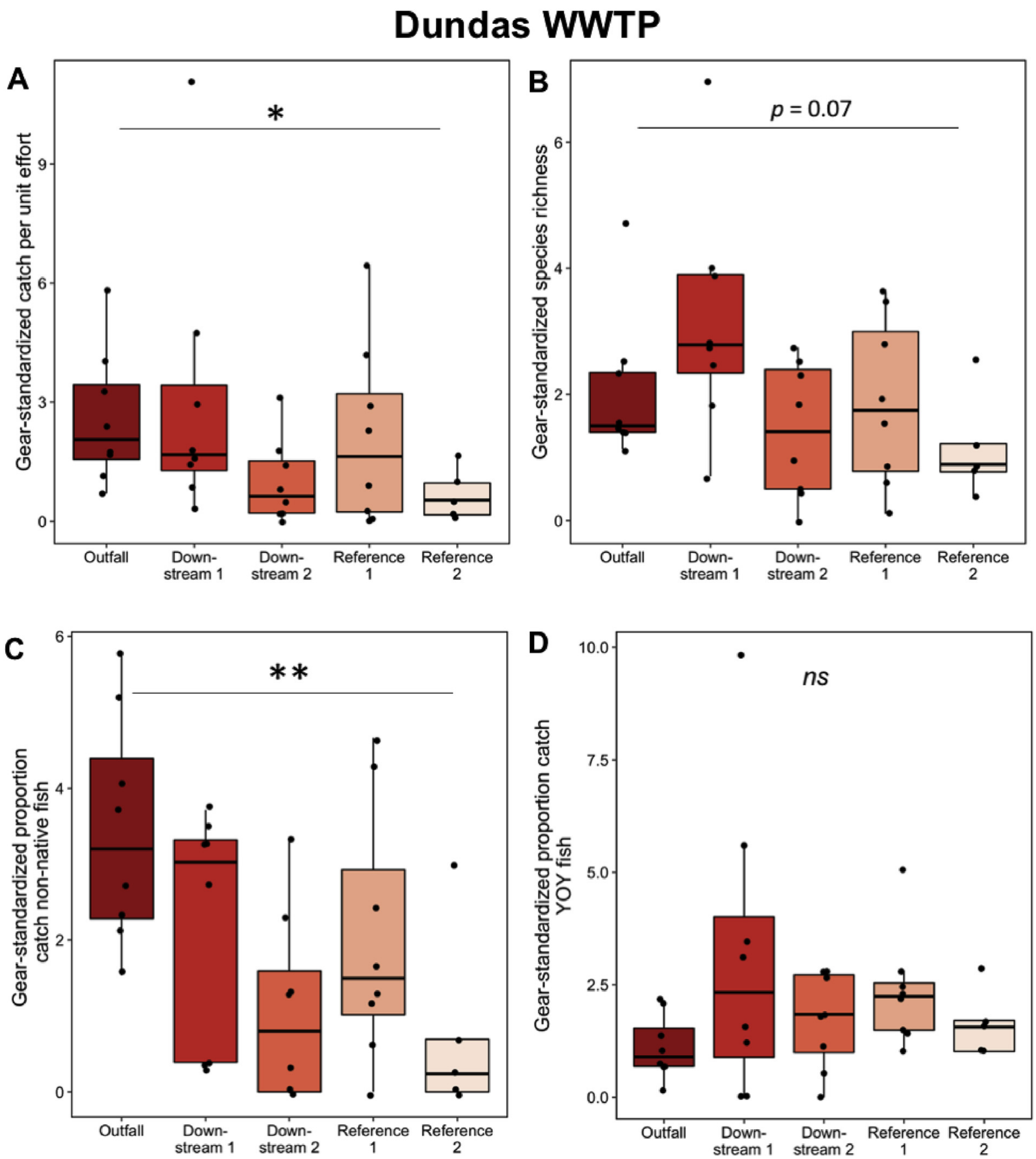

Fig. 2. Boxplots overlaid with data points showing **A)** catch per unit effort (* indicates linear effect, $p < 0.05$), **B)** species richness, **C)** proportion of the catch non-native fish (** indicates linear effect, $p < 0.01$), and **D)** proportion of the catch YOY at sites downstream from the Dundas Wastewater Treatment Plant (WWTP). In all panels, values are mean-standardized across gear collection types to make data comparable. Boxplots show the median and inter-quartile range, whiskers show 1.5*IQR, and points beyond whiskers lie outside 1.5*IQR. "ns" indicates non-significant.

3. Results

3.1. Fish community composition

3.1.1. Dundas WWTP

Across all years, we collected 2297 fish when sampling sites at the Dundas WWTP (661 in 2016, 827 in 2017, and 809 in 2018; Table 1), and this included 25 unique fish species (12 in 2016, 25 in 2017, and 19 in 2018; see Table 1 for full listing). Abundance (catch per unit effort, CPUE) declined with distance from the outfall (Linear model, $N = 37$, Linear term: $t_{30} = -2.34$, $p = 0.026$; Quadratic term: $t_{30} = -0.026$, $p = 0.98$; Fig. 2a). Species richness also tended to decline with distance from the outfall, but this was not statistically significant (Linear model, $N = 37$, Linear term: $t_{30} = -1.85$, $p = 0.075$; Quadratic term: $t_{30} = -0.83$, $p = 0.41$; Fig. 2b). More non-native fish were caught close to the outfall and this declined with distance from the outfall (Linear model, $N = 37$, Linear term: $t_{30} = -2.94$, $p = 0.0063$; Quadratic term: $t_{30} = 0.84$, $p = 0.41$; Fig. 2c). The proportion of YOY fish did not vary across sites (Linear model, $N = 37$, Linear term: $t_{30} = 0.78$, $p = 0.44$;

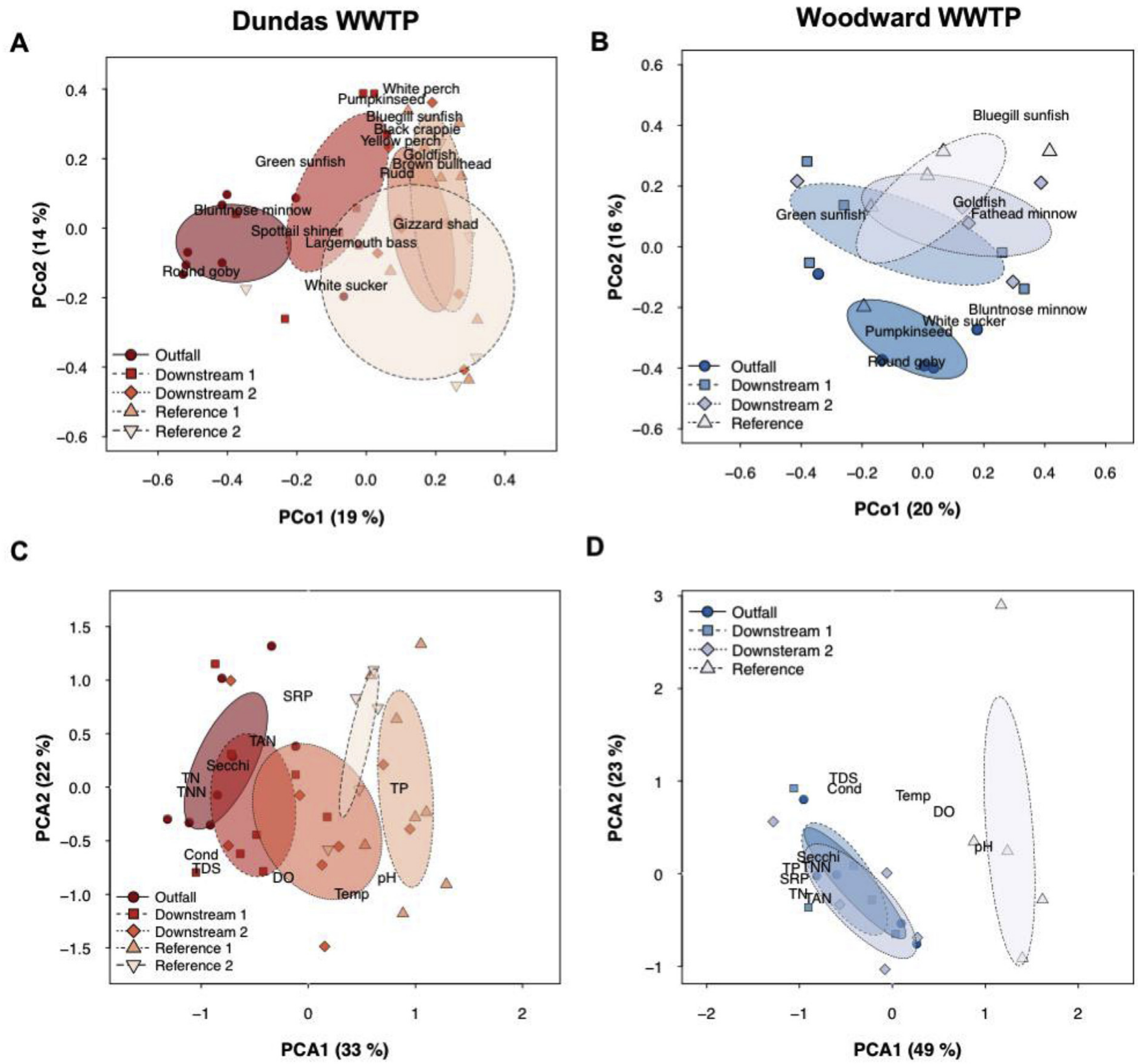

Fig. 3. (**A, B**) Principal coordinate analysis (PCoA) biplots of fish community abundances. Only species where >10 individuals were collected are labelled on the plot, but all species are included in the ordination. (**C, D**) Principal component analysis (PCA) biplots on water quality measures from the Dundas and Woodward Wastewater Treatment Plants (WWTP). For all panels, ellipses are grouped by sampling site and show one standard deviation of variation in the data. Species positions in panels (**A, B**) generated by weighted averages across sites. Graphs were generated with vegan in R and clustered labels were jittered to allow for greater readability.

Quadratic term: $t_{30} = -1.07$, $p = 0.29$; Fig. 2d), but the proportion of tolerant fish declined with increasing distance from the outfall (Linear model, $N = 37$, Linear term: $t_{30} = -2.31$, $p = 0.028$; Quadratic term: $t_{30} = -1.70$, $p = 0.099$). Too few individuals of high resilience (i.e., species with low population doubling times) were collected to analyze if the proportion of resilient fish varied with wastewater exposure ($N = 7$ across all samplings, Table 1). There was no effect of sampling year in any of the above analyses (all contrasts, $p > 0.1$; Fig. S1).

The composition of the fish community differed among sampling sites (Permutation ANOVA$_{Site}$: $F_{(4,30)} = 2.84$, $p < 0.0001$; Fig. 3a). Community composition also tended to differ by year when considered across all sites, but this did not reach statistical significance (Permutation ANOVA$_{Year}$: $F_{(1,15)} = 1.51$, $p = 0.05$; Fig. S2a). The PCoA revealed that the outfall site clustered separately from the remaining sites (Fig. 3a), and sites that were spatially closer to each other tended to cluster together, with the exception of Reference 2 that had a very large representation and indicates a less site-specific community (Fig. 3a). The SIMPER analysis supported this and showed that fish communities were increasingly dissimilar with increasing distance from the outfall (Table 3; for all site contrasts see Supplementary Table S4). Community differences were largely driven by round goby (KW, $\chi^2 = 22.23$, $p < 0.001$) and green sunfish (KW, $\chi^2 = 15.44$, $p = 0.004$) being highly abundant closer to the outfall, while bluegill sunfish were more abundant at reference sites (KW, $\chi^2 = 12.39$, $p = 0.01$), see Table 1, Table 3, Supplementary Table S4. The remaining species that the SIMPER analysis identified as contributing to community differences among sites (yellow perch, gizzard shad, brown bullhead, pumpkinseed) were not statistically different across sites in the species-specific follow up (all KW tests, $p > 0.1$), except goldfish, that were more abundant at reference 1 than reference 2 (KW, $\chi^2 = 10.11$, $p = 0.04$; Supplementary Table S4).

3.1.2. Woodward WWTP

Across all years, we collected 1063 fish from sampling sites at

Table 3
Similarity percentages analysis (SIMPER) showing the contributions of key species (cumulative contribution > 80%) at all sampling sites contrasted with the species found at the outfall sites. Avg. A and Avg. B represents the average catch per unit effort (CPUE, mean-standardized) for each species found at the two sites being compared. See Supplementary Table S4 for full SIMPER site contrasts. Italicized species were also deemed statistically different after KW post-hoc analyses. *indicates borderline significance ($p = 0.06$).

Comparison	Total dissimilarity	Species	Avg A	Avg B	Contribution
Dundas WWTP					
(A) Outfall	75.4%	Round goby	1.87	0.36	38.40
(B) Downstream 1		Green sunfish	0.35	0.29	13.02
		Bluegill sunfish	*0.008*	*0.84*	*12.57*
		Yellow perch	0.08	0.68	9.2
(A) Outfall	91.7%	*Round goby*	*1.87*	*0.00*	*51.96*
(B) Downsteam 2		Green sunfish	0.35	0.09	13.34
		Gizzard shad	0.004	0.22	7.39
(A) Outfall	94.88%	*Round goby*	*1.86*	*0.006*	*43.12*
(B) Reference 1		*Green sunfish*	*0.35*	*0.02*	*11.31*
		Bluegill sunfish	*0.008*	*0.53*	*11.15*
		Goldfish	0.05	0.39	11.03
(A) Outfall	89.30%	*Round goby*	*1.87*	*0.12*	*53.99**
(B) Reference 2		*Green sunfish*	*0.35*	*0.004*	*15.32*
		Yellow perch	0.08	0.24	8.67
Woodward WWTP					
(A) Outfall	82.41%	*Round goby*	*1.86*	*0.09*	*41.49*
(B) Downstream 1		Green sunfish	0.54	1.12	25.62
		Fathead minnow	0.38	0.08	7.02
(A) Outfall	89.63%	*Round goby*	*1.86*	*0.00*	*45.06*
(B) Downstream 2		Green sunfish	0.54	0.23	15.99
		Fathead minnow	0.38	0.37	12.46
		White sucker	0.22	0.07	7.17
(A) Outfall	80.42%	Round goby	1.86	0.17	36.07
(B) Reference		Bluegill sunfish	0.00	0.67	13.53
		Green sunfish	0.54	0.26	12.74
		Fathead minnow	0.38	0.55	12.32

the Woodward WWTP (506 in 2017 and 557 in 2018) and this included 21 unique fish species (13 in 2017 and 21 in 2018; see Table 1 for full species listing). Abundance (CPUE) had a non-linear relationship with proximity to the outfall, as CPUE was highest at the outfall site but was next highest at the reference (Linear model, $N = 20$, Linear term: $t_{15} = -1.90$, $p = 0.07$; Quadratic term: $t_{15} = 2.31$, $p = 0.03$; Fig. 4a). Species richness displayed a similar non-linear relationship with proximity to the outfall as did CPUE (Linear model, $N = 20$, Linear term: $t_{15} = -1.74$, $p = 0.10$; Quadratic term: $t_{15} = 3.53$, $p = 0.003$; Fig. 4b). The proportion of non-native fish did not vary with proximity to the wastewater effluent outfall (Linear model, $N = 20$, Linear term: $t = -0.15$, $p = 0.69$; Quadratic term: $t_{15} = 1.26$, $p = 0.23$; Fig. 4c), nor did the proportion of YOY fish (Linear model $N = 20$, Linear term: $t = 0.31$, $p = 0.76$; Quadratic term: $t_{15} = -0.17$, $p = 0.87$; Fig. 4d). The proportion of tolerant species and the proportion of resilient fish did not vary with proximity to the wastewater effluent outfall (all contrasts, $p > 0.1$). There was no effect of sampling year in any of the above analyses (all contrasts, $p > 0.1$; Fig. S3).

Fish community composition varied across sampling sites (Permutation $ANOVA_{Site}$: $F_{(3,15)} = 1.79$, $p = 0.007$; Fig. 3b) and sampling years (Permutation $ANOVA_{Year}$: $F_{(1,15)} = 2.30$, $p = 0.008$; Fig. S2b). The PCoA revealed that the outfall clustered distinctly from the remaining sites, and there was a high degree of overlap among the remaining sites (Fig. 3b). This was supported by the SIMPER analysis, which indicated that the outfall was most dissimilar from the remaining sites (Table 3). Community differences were largely driven by round goby being highly abundant closer to the outfall (KW, $\chi^2 = 13.09$, $p = 0.004$). In contrast, bluegill sunfish were only collected at the reference site, but the test for across site differences did not reach statistical significance (KW, $\chi^2 = 6.32$, $p = 0.09$; Table 1, Table 3, Supplementary Table S4). The remaining species that the SIMPER analysis identified as contributing to differences among sites (green sunfish, fathead minnow, white sucker) were not statistically different in the species-specific follow up (all KW tests, $p > 0.1$). The two sampling years also clustered separately (Fig. S2b), which was driven, in part, by different species being collected in each year and more unique species being collected in 2018 than in 2017.

3.2. Water quality and habitat characterization

3.2.1. Dundas WWTP

Water quality varied along a gradient as distance from the wastewater treatment plant increased (Permutation $ANOVA_{Site}$: $F_{(4,23)} = 6.20$, $p = 0.0014$; Fig. 3c). Nitrogen (TN, TNN, TAN) and Secchi (water clarity) decreased with distance from the WWTP, while phosphorus (TP, SRP) was highest at downstream 2 and reference 1 (Table 2). Dissolved oxygen was variable but relatively high across sites (all measurements were made during the daytime), and temperature tended to increase by ~2–3 °C with distance from the WWTP (except for reference 2; Table 2). Water quality also varied with year (Permutation $ANOVA_{Year}$: $F_{(2,23)} = 5.0$, $p = 0.008$; Fig. S2c). Sites closest to the wastewater outfall had substantial anthropogenic modifications, while sites further away from the outfall were more natural with little-to-no physical modifications to the shoreline (Supplementary Table S1). The outfall and downstream 1 sites are human-made habitats with evidence of bank erosion, a modified shoreline, and encroaching urban land, while downstream 2, reference 1, and reference 2 were the most natural sampling sites, with unmodified shorelines, wide riparian zones, and neighbouring natural forests and wetland habitat.

3.2.2. Woodward WWTP

All sampling sites downstream from the Woodward WWTP outfall tended to have similar water quality characteristics, while the reference site clustered distinctly (Fig. 3d; Table 1). However,

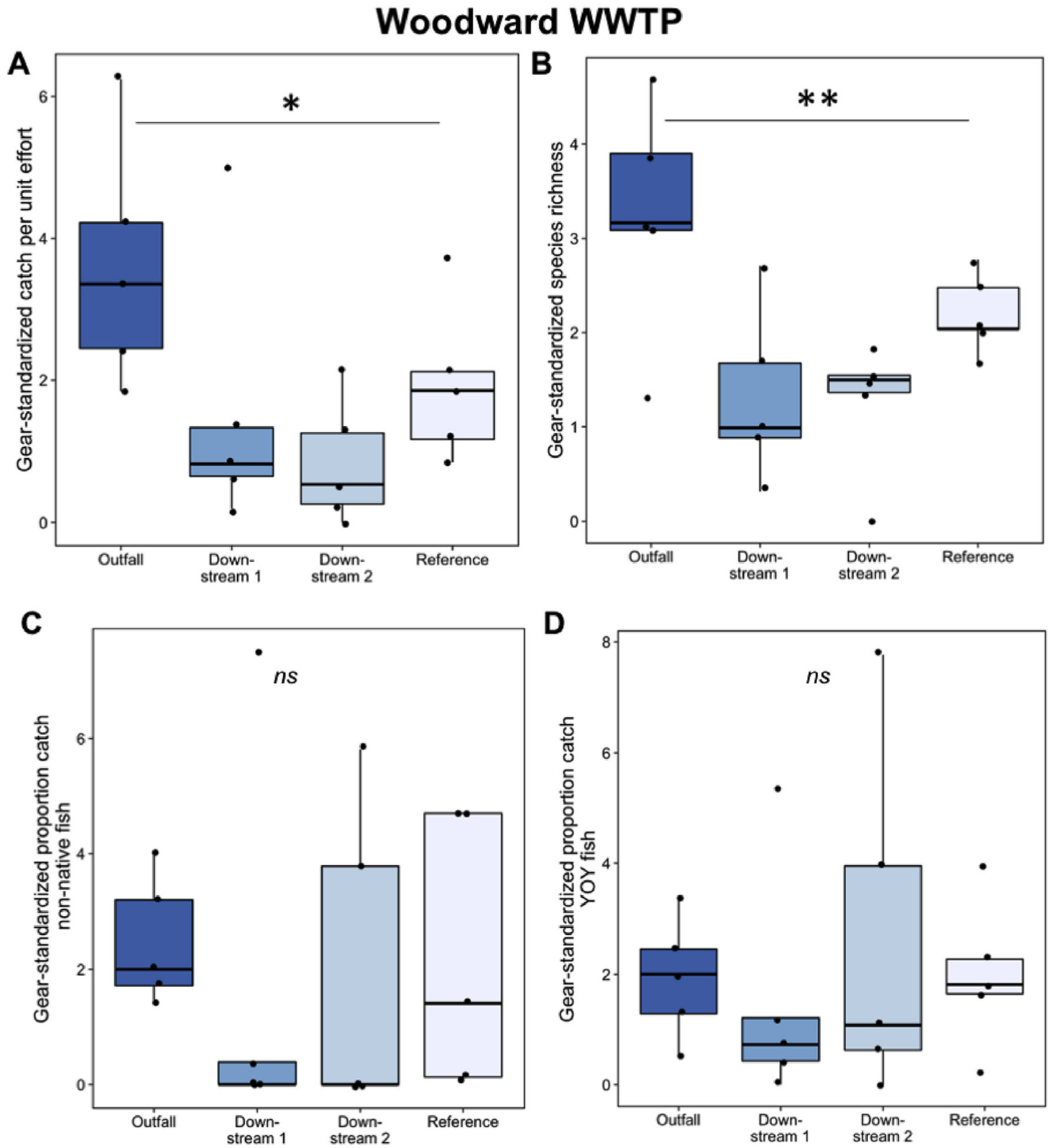

Fig. 4. Boxplots overlaid with data points showing **A)** catch per unit effort (* indicates quadratic effect, $p < 0.05$), **B)** species richness (** indicates quadratic effect, $p < 0.01$), **C)** proportion of the catch non-native fish, and **D)** proportion of the catch YOY at sites downstream from the Woodward Wastewater Treatment Plant (WWTP). In all panels, values are mean-standardized across gear collection types to make data comparable. Boxplots show the median and inter-quartile range, whiskers show 1.5*IQR, and points beyond whiskers lie outside 1.5*IQR. "ns" indicates non-significant.

these differences were not statistically significant (Permutation ANOVA$_{Site}$: $F_{(3,15)} = 0.09$, $p = 0.97$; Fig. 3d). Generally, downstream sites were characterized by relatively high nitrogen, phosphorus (i.e., TN, TAN, TNN, SRP, TP), and Secchi depth (i.e., more clear water) when compared to the upstream reference site. Dissolved oxygen was relatively low at outfall and particularly at the downstream sites. Temperature and pH varied little across the sites and did not follow a gradient of wastewater exposure. Water quality did not vary across years (Permutation ANOVA$_{Site}$: $F_{(1,15)} = 0.59$, $p = 0.46$; Fig. S2d) and there was a high degree of year overlap in the PCA plot (Fig. S2d). All sampling sites, including the upstream reference site were heavily impacted by anthropogenic activity and modifications, had narrow riparian zones, and were bordered by an urban/industrial environment (Fig. 1; Supplementary Table S2).

4. Discussion

We sampled fish communities over 3 years at multiple sites downstream of two distinct WWTPs in Hamilton, Canada. Fish community composition differed along both gradients of wastewater exposure, which supported our initial predictions. A common set of species tended to be more abundant near the WWTP outfalls (i.e., round goby, green sunfish), and both outfall sites had the highest abundances of fish. Species richness and the proportion of non-native and tolerant fish had WWTP-specific trends, with a tendency for fish near WWTP outfalls to be more stress tolerant. In contrast to our predictions, the proportion of YOY fish did not change with wastewater exposure.

The multivariate analysis showed that the fish communities at outfall sites clustered distinctly from the other downstream and reference sites at both WWTPs. At the Dundas WWTP, fish communities tended to become more different with increasing distance from the outfall (i.e., a gradual shift in overlapping community ellipses). This is in contrast to the Woodward WWTP results, where the communities at the downstream and reference sites were more similar to each other and were distinct from the outfall. These findings were supported by the SIMPER analysis, which also broadly showed that all sampling sites were very dissimilar (i.e., high total dissimilarity percentages). It is difficult to provide a general characterization of the fish community responses across both WWTPs because the fish species collected and their relative abundances downstream of the two WWTPs were not similar. Across all sites, more fish species were collected in the Dundas WWTP sampling area than the Woodward WWTP sampling area. The community differences between WWTPs were not compared statistically, but are perhaps not surprising given that these facilities use different treatment technologies (tertiary vs. secondary for Dundas and Woodward, respectively), service different source populations in size and composition (~30 000 residential vs. ~ 480 000 residential/industrial, respectively), and discharge into qualitatively different habitats that would likely support different fish communities in the absence of effluent inputs (wetland vs. creek/riverine; Midwood et al., 2015). The Dundas WWTP sampling area may have more species because it is located in a nature sanctuary and the sampling sites included more habitat types (from dredged channel to a more natural wetland). However, it is important to acknowledge that this wetland is still among the most degraded coastal wetlands in the Great Lakes region (Seilheimer et al., 2011; Thomasen and Chow-Fraser, 2012). Meanwhile, the Woodward WWTP sampling area is entirely urban, industrialized, and human-impacted habitat—even at the reference site. In addition, the Woodward WWTP tended to have higher levels of ammonia (TAN), soluble phosphorus (SRP), and lower dissolved oxygen (especially at the two downstream sites, often < 5 mg/L that is necessary for many fish species; Canadian Council of Ministers of the Environment, 1999) when compared to the Dundas sampling sites.

Even though there were differences in the fish species collected at each WWTP and their downstream habitats, there were certain in signature species that were commonly found near to (or far from) both WWTPs. Previous studies have found that treated wastewater effluent can shape fish communities to be composed of more tolerant or mobile fish (Tetreault et al., 2013) or be dominated by omnivorous species (Ra et al., 2007). Our study supports the former finding, insofar as we provide support for increased tolerant (and non-native) species near the Dundas WWTP, and tolerant species were generally abundant in contaminated sites near both WWTPs. The invasive round goby was one of the most abundant fish collected at both outfalls. Although these sites were characterized by a hard and rocky substrate that round goby are known to prefer (Kornis et al., 2012; Young et al., 2010), so were other sites downstream of WWTPs where round goby were far less abundant (Supplementary Tables S1 and S2). Round goby tolerate a wide range of environmental conditions (e.g., low dissolved oxygen, variable salinities; Kornis et al., 2012), and in a previous study they showed no behavioural or physiological effects following a month-long in-situ wastewater exposure at the Dundas WWTP (McCallum et al., 2017a). Round goby therefore appear able to tolerate the environmental conditions at the outfall, and may either occupy a previously empty niche or outcompete other species for this space. Green sunfish were also abundant at both outfall sites and downstream sites, and have previously been collected in wastewater-impacted sites in the Great Lakes region (Reash and Berra, 1987). In contrast, bluegill sunfish were more abundant at reference sites for both WWTPs. This aligns with previous work showing that bluegill sunfish have poor survival and suffer a significant metabolic cost with increasing exposure to wastewater effluent (Du et al., 2018). Long-lived, top-predator species (e.g., bowfin, northern pike, longnose gar), which are frequently targets for restoration works in Hamilton Harbour (Boston et al., 2016), were rarely encountered in our study collections and were never collected at the outfall sites or immediately downstream.

Another measure that similarly increased near both WWTPs was fish abundance: fish were most abundant at the outfall sites, indicating they may be attracted to the effluent or outfall habitat. Nutrients and/or organic particulate matter may directly attract fish or indirectly increase food/resource availability (e.g., aquatic invertebrates) which increases the carrying capacity at these sites *via* a “bottom-up” effect. For example, both Azzurro et al. (2010) and Brown et al. (2011) found increased fish abundances near wastewater outfalls in the Mediterranean Sea and the Speed River (Canada), respectively. Another non-mutually exclusive reason that fish may be attracted to the outfall is because the effluent is thermally stable and it may provide refuge from unseasonably high or low temperatures. Indeed, temperatures at the two outfall sites in our study showed the least variance over time (See Table 2 for temperature standard deviations and ranges). It would be beneficial to measure fish communities across the entire season, including the winter, to assess whether some species use the outfall as a refuge all year. The increases in fish abundance that we observed closer to WWTPs in the summer may be magnified in the winter, as fish are likely to seek out deeper, thermal refugia with warmer and more stable temperatures (Caissie, 2006). Additionally, macroinvertebrate sampling could be conducted along the exposure gradient to address resource availability across sites, which together would be beneficial to address why fish are attracted to outfall sites. Regardless of the mechanism of attraction, wastewater outfalls may act as an ecological trap for fish (Irwin, 2004; Schlaepfer et al., 2002), where they are inadvertently exposed to increased levels of anthropogenic pollution being discharged in the treated effluent (Csiszar et al., 2011; McCallum et al., 2017a, 2017b; Muir et al., 2017), which may be detrimental to their physiology, reproduction, and survival (Holeton et al., 2011).

Sampling year did not affect fish abundance, species richness, and the proportion of non-native, tolerant, resilient, or YOY fish. However, the multivariate analysis indicated that fish communities did vary across years at the Woodward WWTP, while the community ellipses showed a high degree of overlap across years for the Dundas WWTP (Supplementary Materials Fig. S1). The yearly variation may be driven in part by variation in environmental conditions. For example, 2016 was characterized by a drought with extremely low water levels, while 2017 was characterized by record high water levels in this ecosystem (Environment Canada, 2019). Variation in water level can concentrate or dilute the effluent and any associated effects it has on water quality, fish, and their communities. Year-to-year differences were not a primary focus of this study, but should be acknowledged and considered when quantifying and mitigating how wastewater effluent shapes fish communities.

This study is one of only a few to quantify the effects of wastewater effluent on fish communities across multiple years downstream from multiple WWTPs. Despite differences between the source populations, wastewater treatment approaches and receiving habitats of the two WWTPs studied here, we found that fish were attracted to the outfall sites and certain “signature” species were common at both outfalls. It would be beneficial to identify why fish are attracted to effluent and investigate whether it acts as an ecological trap by negatively affecting their survival or reproduction. Such research would help mitigate fish exposure to effluent-associated pollutants in the wild and restore fish communities in habitats receiving effluent. As human populations grow in urban areas, so too will the volume of treated wastewater that is discharged into surface waters. Our results suggest that this may have an impact on fish communities, an understanding of which is critical for managing and protecting freshwater resources.

Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Summary statement

Fish communities diverged along a gradient of wastewater exposure near two wastewater treatment plants. Fish were also more abundant near the effluent outfalls.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.06.075.

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