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Invertebrate prey availability, habitat condition and Redside Dace (*Clinostomus elongatus*) status in Greater Toronto Area streams

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Redside Dace (*Clinostomus elongatus*) is a small, colorful cyprinid found in pools and slow-flowing sections of Ontario streams. As a result of recent declines and threats to remaining populations, the species was assessed as Endangered in Canada. Habitat degradation caused by urban development is considered a primary threat. However, a clearer understanding of associated impacts is required to inform assessments of population trajectories and habitat supply. In this study, we compared riparian vegetation, stream habitat, and terrestrial (riparian) and aquatic invertebrate abundance and diversity among 24 Greater Toronto Area (GTA) sites that represented three population status categories of Redside Dace (extirpated, declining or stable). Sites were sampled during the summers of 2008 and 2009. Stream habitat was characterized using the Ontario Stream Assessment Protocol (OSAP) rapid assessment methodology (RAM). Invertebrate prey availability was characterized using drift samplers and sweep nets. Clear separation of population groups was evident using multivariate analysis of riparian vegetation and bank stability data. However, significant univariate differences were only identified for riparian vegetation; with greater amounts of grasses at stable population sites and more bare ground at extirpated sites. Using instream habitat data (e.g., amount of riffles and pools), there was less separation of population groups in multivariate space. For both terrestrial and aquatic invertebrate data sets, abundance, biomass and taxa diversity did not significantly differ among groups. Multivariate differences in taxa composition were also not significant. Small sample sizes and high data variability limited our ability to detect significant instream habitat and invertebrate differences among groups. Other factors that may have influenced study results include high regional variation in surficial geology and topography, habitat and invertebrate sampling methods, and differing responses of Redside Dace and invertebrate taxa to environmental stressors.

INTRODUCTION

Redside Dace (*Clinostomus elongatus*) is a small, colorful cyprinid found in the pools and slow-flowing sections of small streams with a mixture of overhanging grasses and shrubs, and pool and riffle habitats (McKee and Parker 1982, Novinger and Coon 2000). The species demonstrates a strong preference for mid-water positions in the deepest parts of pools (Novinger and Coon 2000). Redside Dace has a disjunct distribution across North America throughout the upper Mississippi River Drainage, Great Lakes Basin, Ohio River and upper Susquehanna River (Page and Burr 1991). As a result of recent declines and threats to remaining populations, the species has been assessed as Endangered in the province of Ontario (OMNRF 2016) and in Canada (COSEWIC 2017). Redside Dace is thought to be extirpated from almost half of the historically occupied watersheds in Canada, with many remaining populations in decline (COSEWIC 2007).

Almost 80 percent of its Canadian distribution is located within the boundaries of the City of Toronto and adjacent municipalities. In the Greater Toronto Area (GTA), the human population is expected to grow by 37% by 2031 (MPIR, 2004), and therefore the stresses affecting remaining Redside Dace populations can be expected to increase. Habitat degradation caused by urban development activities is considered a primary threat facing Canadian Redside Dace populations (COSEWIC 2007); with local population sizes negatively affected by increasing amounts of upstream urban land-use (Poos et al. 2012). Increased siltation, removal of riparian vegetation, channelization, pollution, and altered stream hydrology are considered detrimental to Redside Dace (McKee and Parker 1982, Reid and Parna 2017).

Redside Dace is a specialized feeder (insectivore) with a diet dominated by terrestrial invertebrates; Diptera (flies) comprise a large component of prey consumed (Schwartz and Norvell 1958, McKee and Parker 1982, Daniels and Wisniewski 1994). Aquatic drifting invertebrates represent a smaller component of the diet. In order to capture terrestrial invertebrates, Redside Dace jump out of the water to capture prey. Clear water conditions are considered important for visually detecting prey flying (or swarming) above the water's surface (Daniels and Wisniewski 1994). Overhanging riparian vegetation also provides important contributions of terrestrial invertebrates to the diet of Redside Dace (McKee and Parker 1982, Andersen 2002) and cover from predation (Novinger and Coon 2000).

Across North America, urbanization has been shown to have a negative impact on the density, richness and composition of stream benthic invertebrate assemblages (O'Driscoll et al. 2010). Invertebrate taxa richness and the prevalence of pollution sensitive groups (e.g., mayflies, stoneflies and caddisflies) are greatly reduced in heavily urbanized (>50% percent land cover) Lake Ontario watersheds; where tolerant species (e.g., chironomids) dominate (Stanfield and Kilgour 2006, Bazinet et al. 2010). These responses are interpreted to reflect factors such as winter road salt application (high chloride concentrations), highly altered flow conditions, and limited amounts of upland forest cover. These past studies suggest that prey availability for Redside Dace populations in urbanizing Ontario watersheds may have declined. For stream-dwelling fishes, experimental and modelling-based studies have demonstrated that low aquatic invertebrate prey availability negatively affects growth and body condition, and that resource subsidies from terrestrial sources are essential for meeting energetic requirements (Simpkins and Hubert 2000, Sweka and Hartman 2008, Eros et al. 2012, Akbaripasand et al. 2014).

A priority Redside Dace recovery action is to identify key factors (threats) associated with urban development that contribute to population declines (RDRT 2010). It is anticipated that this knowledge will improve the ability of resource managers to protect and enhance Redside Dace habitat through urban planning and the use of best management practices. A clearer understanding of the impacts of urban development is also required to inform assessments of population trajectories, and the degree to which supply of suitable habitat meets the current and future needs of the Redside Dace. In this study, we compared riparian vegetation composition, stream habitat characteristics, and terrestrial (riparian) and aquatic invertebrate abundance and diversity among GTA sites representing three population status categories of Redside Dace (extirpated, declining or stable). The overall objective of the research was to determine whether differences in population status among sites correspond to differences in habitat condition and invertebrate prey availability.

METHODS

Twenty-four stream sites across the GTA (Figure 1), eight within each Redside Dace population status category, were sampled once during the summer of 2008 or 2009. Sites were categorized as extirpated, declining or stable. Population status was assigned based on historical and recent Redside Dace distribution and abundance information provided in Anderson (2002), COSEWIC (2007), Reid et al. (2008) and Poos et al. (2012). Sites were located in the following watersheds: Credit River, Don River, Fourteen Mile Creek, Humber River, Morrison Creek, Petticoat Creek, and Rouge River. The channel width at each site ranged between 1.1 and 6.1 m wide (median = 3.2 m). Surrounding and upstream urban landcover varied greatly among the study sites (Figure 1). Extirpated population sites were located in heavily urbanized watersheds, while almost all stable population sites were located outside urban areas. It is recognized that based on the recent COSEWIC status reassessment (COSEWIC 2017), the population status of several study sites has deteriorated since 2009.

*Figure 1. Distribution of habitat and invertebrate sampling sites (n=24) across the Greater Toronto Area (GTA). Sites were sampled in the summers of 2008 and 2009. Yellow dots represent declining, red extirpated and green as stable status for Redside Dace (*Clinostomus elongatus*) populations.*

HABITAT SAMPLING

Stream habitat was characterized using the Ontario Stream Assessment Protocol (OSAP) rapid assessment methodology (RAM; Stanfield 2005). RAM is a visually based, point transect survey method for characterizing in-stream habitat. At each site, habitat was characterized at 60 points along 10 to 30 evenly spaced transects. Site length was 100 m. Depending on channel width, two to six points were characterized along each transect. Habitat characteristics described at each site include:

1. percent composition of pools, glides, and riffles in relation to the total amount of habitat present,
2. amount of in-stream cover (flat rock, round rock, wood, macrophytes, banks, or other in-stream material),
3. point substrate size-classes (fines: < 2mm diameter; gravel: 2 to 100 mm; cobble: 101 to 1000 mm, bedrock > 1000 mm), and
4. bank stability (eroding, vulnerable, protected, or depositional).

Hydraulic head (the height to which water climbs a ruler held at right angles to flow) was used as a surrogate for water velocity and the basis for identifying pools (0 to 3 mm), glides (4 to 7 mm), and riffles (>7 mm). Pools and glides were stratified into three depth categories: shallow (<100 mm); medium (100 to 600 mm), and deep (> 600 mm).

Riparian vegetation was characterized on each side of the stream at 10 m intervals evenly distributed along 100 m transects. Assessments were completed within a 1 m^2 plot located 1 to 2 m from the stream bank. Within each plot, percent coverage of the following plant categories was visually assessed:

1. bare ground;
2. grasses, weeds, sedges, forbs;
3. shrubs;
4. deciduous trees; and,
5. coniferous trees.

The presence of overhead tree cover at each plot was also noted.

AQUATIC AND TERRESTRIAL INVERTEBRATE SAMPLING

Previous southern Ontario urbanization-impact studies used benthic invertebrate data collected by kick net sampling the streambed (Stanfield and Kilgour 2006, Bazinet et al. 2010). In this study, the composition of invertebrate prey availability was characterized using drift samplers and sweep nets (Daniels and Wisniewski 1994).

At each site, five 1-hour aquatic drift samples (during daylight hours) were collected using drift nets placed in the transition zone between riffles and deeper pool/run habitats. Drift nets were set with 2 to 3 cm of the opening (dimensions: 46 cm long x 31 cm wide) remaining above the water surface to sample surface drift. Water velocity was measured with a Swoffer™ water velocity meter immediately upstream in the mid column of the drift nets. Terrestrial invertebrates were also collected from the riparian vegetation using a standardized sweep net method. Along six of the 10-m riparian vegetation assessment transects, invertebrates were collected with a sweep net (38 cm diameter hoop) from the riparian vegetation for 1 minute (Buffington and

Redak 1998). Aquatic invertebrate samples were preserved in labelled vials with 70% ethanol. Sweep net samples were individually stored in Ziploc bags and kept frozen until processing.

Aquatic and terrestrial invertebrates were identified to family with the aid of a Nikon SMZ 1500 microscope (Lehmkuhl et al. 1979, Smith 2001, Marshall 2006). It was assumed that higher-level taxonomic identification (*i.e.,* genus-level) would not improve the detection of community-level differences among sites (Bowman and Bailey 1997). Each specimen was also identified to life stage (i.e., larva, nymph, adult), and enumerated. Biomass of each sweep or drift net sample was determined by drying samples at 60°C for 24 to 48 hrs, and measuring the dry weight of each sample with a digital balance (to nearest g). Although samples from each site were processed individually, data was pooled prior to analysis to provide site-level values for aquatic and terrestrial invertebrates.

DATA ANALYSIS

Sample site locations were not selected randomly but chosen based on prior knowledge of Redside Dace population status. Some sites are in close proximity, and therefore associated data could be spatially autocorrerelated (Legendre 1993). Prior to analysis, spatial autocorrelation of population status among sampling sites was assessed using a Mantel test to establish site independence. A matrix of the linear distances between sites based on latitude-longitude was derived. Population status was coded as: extirpated = 1, declining = 2, stable = 3. A distance matrix was then constructed based on the site categorization. The correlation between the two matrices was tested by using the 'mantel.rtest' function in the 'ade4' package in R (R Core Team) with 9999 permutations. Redside Dace population status across sampling sites was not spatially auto-correlated (Mantel test: $r = 0.09$, $P = 0.09$).

Differences in the total number, total biomass and taxa diversity of invertebrates sampled from sites representing different population status were tested using Analysis of Variance (ANOVA). Prior to analysis, the number and biomass of invertebrates collected by drift sampling were standardized by volume of water (*i.e.,* flow) that passed through the drift net during the sample. Also, given their importance as a Redside Dace prey, among group differences in the number of adult Diptera (flies) and Empididae (danceflies) collected by sweep nets were tested. Taxa diversity was determined for each site using the Shannon index ($H = -sum\ p_i\ log(b)\ p_i$) where p_i is the proportion of taxa i and b is the base of the logarithm. Taxa evenness was determined for each site using Pielou's evenness index ($J = H / \ln(S)$), where S is the total number of taxa observed. ANOVA was also used to test for univariate habitat differences among population status groups. To meet the assumptions of an ANOVA, abundance and biomass data were $\log_{10}$ transformed, and percent-based habitat data were arc-sin transformed.

Multivariate methods were used to test:

1. for compositional differences in terrestrial and aquatic macroinvertebrate samples among Redside Dace population status groups;
2. whether habitat characteristics can predict population status; and
3. whether habitat characteristics can explain the among site variation in the composition of invertebrate samples.

Details of multivariate analyses are presented below.

Multi-response permutation procedure (MRPP) was used to test whether the composition of terrestrial and aquatic invertebrate samples (done separately) differed among Redside Dace status groups. The Bray-Curtis distance metric was used to measure the dissimilarity among groups. Significance was assessed by using a Monte-Carlo permutation with 9999 iterations using the function ‘mrpp’ in the ‘vegan’ package of R. Differences among sites in multivariate space were visualized using non-metric dimensional scaling (nMDS), and the Bray-Curtis measure. Analyses were conducting using software PC-ORD V.5.10.

Indicator species analysis (ISA) was conducted to identify individual taxa that may be associated with different Redside Dace population status groups. Analysis was run separately for terrestrial invertebrates and aquatic invertebrates. The ‘multipatt’ function in the R package ‘indicspecies’ was used with a maximum order of site group combinations set at 3 (for each status group) and 5000 permutations to estimate p. Terrestrial and aquatic invertebrate taxa abundances were ranked using the ‘rankabundance’ function in the Biodiversity package in R to identify the most prevalent species.

Discriminant function analysis (DFA) was used to test how well instream habitat characteristics, and riparian vegetation and bank stability data clustered sites into Redside Dace population status groups, and to identify influential habitat characteristics. Habitat variables were standardized prior to analysis by subtracting the mean value and dividing by the standard deviation (Legendre and Legendre 1998). Classification success was evaluated using a jackknife method. Statistical analysis was conducted in R using the package ‘MASS’.

The relative influence of habitat variables (instream and riparian separately) on invertebrates (aquatic and terrestrial separately) was assessed using Canonical correspondence analysis (CCA). A Monte Carlo test was used to test the significance of each CCA model. Analyses were conducting using software PC-ORD V.5.10.

RESULTS

STREAM AND RIPARIAN HABITAT

Univariate habitat differences among Redside Dace population status groups were evident; with the greatest contrasts between stable and extirpated populations (Table 1 and 2). In comparison to extirpated populations, greater amounts of fine sediment, shallow pool and glide habitats, instream cover, riparian grasses, and vulnerable banks were measured at stable sites. Conversely, at extirpated sites, greater amounts of cobble and bedrock, medium-depth and deep pools, bare ground, overhead tree cover, and protected banks were measured. Of these variables, among-group differences were only significant for bare ground (ANOVA: $F = 7.6$, $p = 0.003$), riparian grasses (ANOVA: $F = 9.7$, $p = 0.001$), and overhanging tree cover (ANOVA: $F = 6.9$, $p = 0.005$).

Based on riparian vegetation and bank stability data, discriminant function analysis indicated a significant difference among the population status groups ($p = 0.013$) (Figure 2a). Ninety percent of variation among the groups was explained by the first discriminant function, and *a posteriori* classification accuracy using the discriminant function ranged from moderate to high for each group (63-75%). Habitat variables contributing most substantially to the first discriminant axis were the percent coverages of grasses, bare ground, and overhead tree cover (Table 2). Using instream habitat data, there was greater overlap among the three population categories (Figure 2b) and discriminant functions were not significant ($p = 0.32$).

Table 1. Riparian habitat, and bank stability-based comparison of 24 Redside Dace sites (grouped by population status). Sites were sampled in the summers of 2008 and 2009. STD = standardized discriminant coefficient from first discriminant function.

Habitat	Mean (Standard Deviation)			
	Extirpated (n=8)	Declining (n=8)	Stable (n=8)	STD
Riparian Vegetation				
Bare ground	37.6 (21.5)	21.8 (15.5)	8.5 (5.7)	-0.387
Grasses	50.8 (18.2)	71.5 (12.6)	82.5 (10.7)	0.466
Shrubs	10.3 (8.9)	4.3 (4.7)	7.5 (7.5)	-0.08
Deciduous trees	1.3 (1.6)	1.5 (2.1)	1.3 (1.3)	-0.003
Coniferous trees	0.0	0.16 (0.4)	0.4 (1.2)	-0.007
Overhead cover	73.8 (23.9)	66.3 (19.2)	34.4 (19.5)	-0.391
Bank Stability				
Eroding	24.5 (25.2)	33.3 (22.6)	33.3 (30.3)	0.07
Vulnerable	0.5 (1.1)	27.1 (25.3)	23.0 (30.9)	0.203
Protected	32.8 (32.7)	18.5 (21.3)	14.5 (16.6)	-0.155
Depositional	2.0 (2.2)	1.4 (2.3)	1.9 (5.3)	-0.007

Table 2. Instream habitat-based comparisons of 24 Redside Dace sites (grouped by population status). Sites were sampled in the summers of 2008 and 2009. STD = standardized discriminant coefficient from first discriminant function.

Habitat	Mean (Standard Deviation)			
	Extirpated (n=8)	Declining (n=8)	Stable (n=8)	STD
Channel Width				
Total	4.2 (1.7)	3.3 (1.5)	3.0 (1.4)	-0.244
Substrate				
Fines	43.8 (24.9)	61.7 (10.9)	58.5 (18.5)	0.261
Gravel	28.9 (12.1)	29.4 (11.8)	24.3 (13.2)	-0.092
Cobble	19.4 (22.2)	8.9 (12.3)	15.5 (17.0)	-0.096
Bedrock	7.9 (17.6)	0.0	1.7 (3.4)	-0.201
Fines	43.8 (24.9)	61.7 (10.9)	58.5 (18.5)	0.261
Habitat				
Small pool	16.1 (23.4)	23.9 (18.2)	35.2 (21.8)	0.25
Small glide	1.8 (3.0)	9.2 (6.6)	8.8 (11.7)	0.288
Medium pool	51.2 (19.5)	35.6 (16.8)	36.6 (17.6)	-0.265
Medium glide	11.8 (12.6)	19.4 (26.8)	8.6 (8.4)	-0.016
Large pool	9.3 (16.1)	1.9 (4.7)	1.9 (3.3)	-0.24
Riffle	11.3 (12.8)	8.0 (9.7)	11.4 (10.4)	-0.017
Cover	8.1 (8.2)	24.1 (20.7)	28.9 (35.9)	0.26

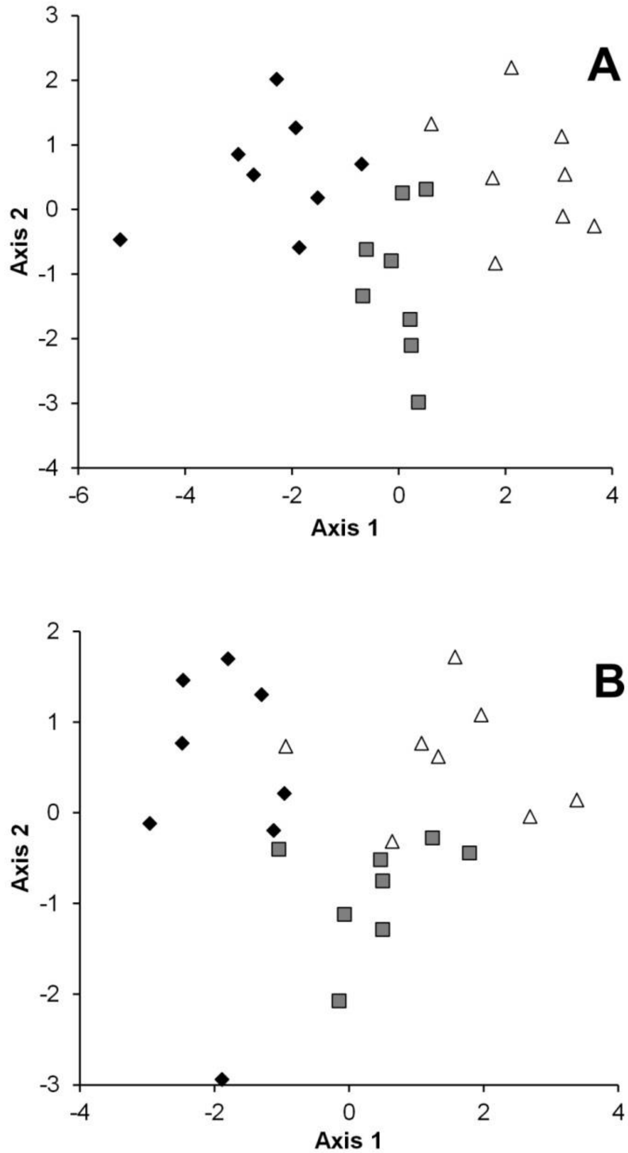


Figure 2. Bi-plots of habitat-based discriminant function analysis (DFA) site-scores (n=24) associated with three Redside Dace population status groups (stable Δ; declining ■; and extirpated ♦). Analysis was done separately for riparian vegetation and bank stability (A) and instream habitat (B) data.

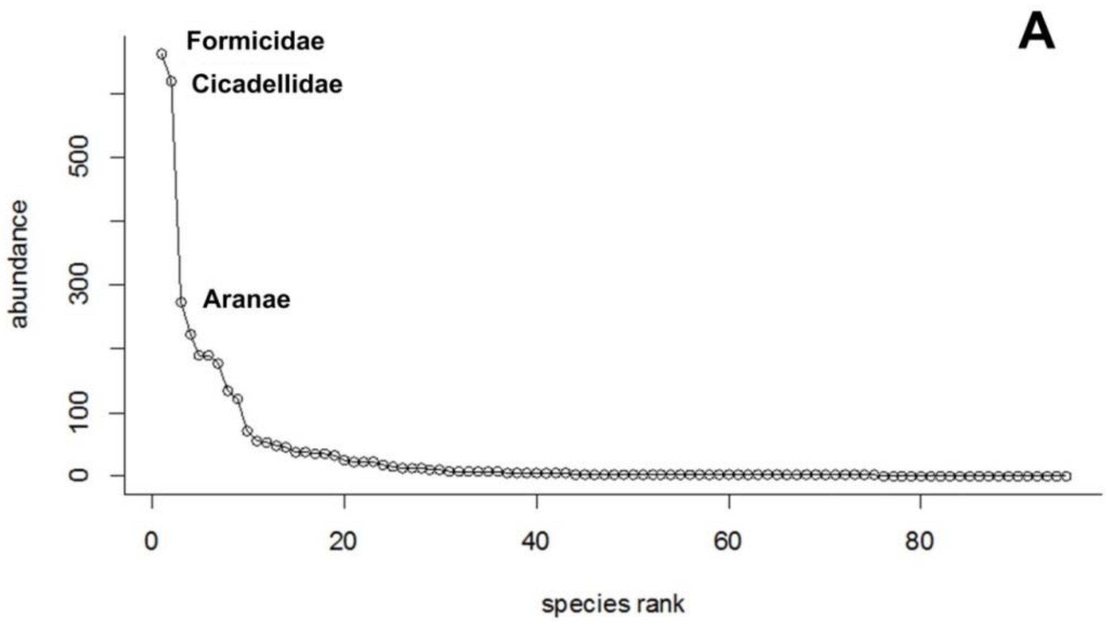
AQUATIC INVERTEBRATES

Drift nets collected a total of 2968 invertebrates that were identified to 81 families (or orders when unable to key out further). Formicidae (ants, 45.5% of individuals collected), Chironomidae (non-biting midges) larvae (13.7%) and Chironomidae adults (4.6%) were the most abundant macroinvertebrates in the drift nets (Table 3, Figure 3b). The largest difference among Redside Dace population groups was the greater biomass of drifting aquatic invertebrates collected from stable sites (Table 4). However, mean abundance and biomass of aquatic invertebrates did not differ significantly among groups (ANOVA: abundance, $F = 0.001$, $p = 0.98$; biomass, $F = 2.89$, $p = 0.10$). Similarly, taxa diversity, richness or Pielou's evenness did not significantly differ among groups (ANOVA: $F = 1.57$, $p = 0.22$; $F = 0.003$, $p = 0.96$; $F = 0.16$, $p = 0.69$ respectively).

Table 3. Invertebrate taxa collected by aquatic drift nets at 24 sites over a range of Redside Dace population status.

Phylum	Class	Order	Taxa	Stable	Declining	Extirpated
Annelida	Clitellata	Arhymchlodellida	Arhymchlodellida		+	
Annelida	Clitellata	Arhynchobdellida	Hirudinae		+	+
Annelida	Clitellata	Oligochaeta	Oligochaeta	+	+	+
Arthropoda	Arachnida	Araneae	Araneae(order)	+	+	+
Arthropoda	Arachnida	Trombidiformes	Hydracarina	+	+	
Arthropoda	Arachnida	Trombidiformes	Hygrobatoidae	+	+	+
Arthropoda	Chilopoda	Trombidiformes	Chilopoda	+		
Arthropoda	Diplopoda	Spirobolida	Spirobolidae	+	+	
Arthropoda	Entognatha	Collembola	Collembola			+
Arthropoda	Entognatha	Entomobryomorpha	Entomobryidae	+		+
Arthropoda	Entognatha	Entomobryomorpha	Isotamidae	+	+	+
Arthropoda	Insecta	Coleoptera	Chrysomelidae	+	+	+
Arthropoda	Insecta	Coleoptera	Cleridae			+
Arthropoda	Insecta	Coleoptera	Coccinelidae		+	+
Arthropoda	Insecta	Coleoptera	Coleoptera	+	+	+
Arthropoda	Insecta	Coleoptera	Curculionidae	+	+	+
Arthropoda	Insecta	Coleoptera	Dytiscidae	+		+
Arthropoda	Insecta	Coleoptera	Elateridae		+	+
Arthropoda	Insecta	Coleoptera	Elmidae	+	+	+
Arthropoda	Insecta	Coleoptera	Haliplidae	+		+
Arthropoda	Insecta	Coleoptera	Histeridae	+		
Arthropoda	Insecta	Coleoptera	Hydrophilidae	+		+
Arthropoda	Insecta	Coleoptera	Lymexylidae		+	+
Arthropoda	Insecta	Coleoptera	Noteridae		+	
Arthropoda	Insecta	Coleoptera	Passandridae		+	
Arthropoda	Insecta	Coleoptera	Ptilodactylidae			+
Arthropoda	Insecta	Diptera	Ceratopogonidae	+		+
Arthropoda	Insecta	Diptera	Chaoboridae			+
Arthropoda	Insecta	Diptera	Chironomidae	+	+	+
Arthropoda	Insecta	Diptera	Culicidae	+	+	+
Arthropoda	Insecta	Diptera	Diptera	+	+	+
Arthropoda	Insecta	Diptera	Dixidae	+	+	+
Arthropoda	Insecta	Diptera	Dolichopodidae		+	+
Arthropoda	Insecta	Diptera	Empididae		+	+
Arthropoda	Insecta	Diptera	Muscidae	+		+
Arthropoda	Insecta	Diptera	Nematocera		+	
Arthropoda	Insecta	Diptera	Phoridae		+	
Arthropoda	Insecta	Diptera	Psychodidae	+		+
Arthropoda	Insecta	Diptera	Scenopinidae			+
Arthropoda	Insecta	Diptera	Simuliidae	+	+	+
Arthropoda	Insecta	Diptera	Tabanidae			+
Arthropoda	Insecta	Diptera	Therevidae			+
			Tipulidae	+	+	

Phylum	Class	Order	Taxa	Stable	Declining	Extirpated
Arthropoda	Insecta	Ephemeroptera	Baetidae	+	+	+
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae			+
Arthropoda	Insecta	Ephemeroptera	Ephemeroptera		+	+
Arthropoda	Insecta	Ephemeroptera	Heptageniidae		+	
Arthropoda	Insecta	Hemiptera	Aradidae		+	
Arthropoda	Insecta	Hemiptera	Cicadellidae	+		+
Arthropoda	Insecta	Hemiptera	Corixidae	+		+
Arthropoda	Insecta	Hemiptera	Gerridae	+	+	
Arthropoda	Insecta	Hemiptera	Hemiptera	+	+	+
Arthropoda	Insecta	Hemiptera	Heteroptera	+		
Arthropoda	Insecta	Hymenoptera	Apidae		+	
Arthropoda	Insecta	Hymenoptera	Braconidae		+	
Arthropoda	Insecta	Hymenoptera	Crabronidae		+	
Arthropoda	Insecta	Hymenoptera	Dryinidae	+	+	+
Arthropoda	Insecta	Hymenoptera	Formicidae	+	+	+
Arthropoda	Insecta	Hymenoptera	Halictidae		+	
Arthropoda	Insecta	Hymenoptera	Hymenoptera		+	+
Arthropoda	Insecta	Hymenoptera	Vespidae	+	+	+
Arthropoda	Insecta	Lepidoptera	Arctiidae		+	
Arthropoda	Insecta	Lepidoptera	Lepidoptera	+		+
Arthropoda	Insecta	Lepidoptera	Noctuidae	+		
Arthropoda	Insecta	Lepidoptera	Sphingidae			+
Arthropoda	Insecta	Odonata	Gomphidae		+	
Arthropoda	Insecta	Orthoptera	Acrididae	+	+	+
Arthropoda	Insecta	Plecoptera	Plecoptera	+		+
Arthropoda	Insecta	Thysanoptera	Thripidae			+
Arthropoda	Insecta	Trichoptera	Hydropsychidae	+	+	+
Arthropoda	Insecta	Trichoptera	Hydroptilidae		+	
Arthropoda	Insecta	Trichoptera	Polycentropodidae	+	+	+
Arthropoda	Malacostraca	Amphipoda	Amphipoda	+		
Arthropoda	Malacostraca	Amphipoda	Gammaridae	+	+	
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	+	+	+
Arthropoda	Malacostraca	Isopoda	Asellidae	+	+	+
Arthropoda	Ostracoda	Isopoda	Ostracoda			+
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	+		+
Mollusca	Gastropoda	Basommatophora	Physidae	+	+	+
Mollusca	Gastropoda	Hygrophila	Planorbidae	+	+	+



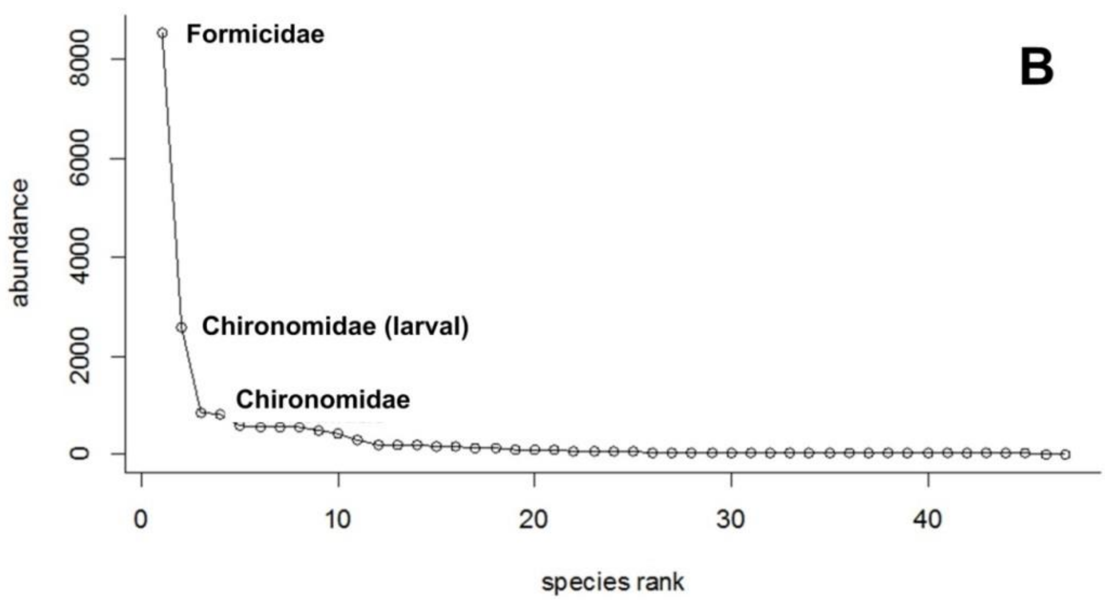


Figure 3. Rank-abundance plots for (A) terrestrial invertebrates and (B) aquatic invertebrates collected from 24 Redside Dace sampling sites. Aquatic invertebrate data was standardized by stream flow.

Table 4. Invertebrate-based comparisons of 24 Redside Dace sites (grouped by population status). Aquatic invertebrates were sampled with drift nets and terrestrial invertebrates were sampled with sweep nets. Sites were sampled in the summers of 2008 and 2009.

	Mean (Standard Deviation)		
	Extirpated (n=8)	**Declining (n=8)**	**Stable (n=8)**
Abundance (individuals)			
Aquatic drift[1]	723.9 (616.7)	872.6 (1359.6)	788.7 (859.7)
Riparian sweep	113.4 (60.6)	115.5 (71.6)	205.0 (116.7)
Biomass (g)			
Aquatic drift[1]	0.4 (0.3)	0.8 (0.8)	1.3 (1.7)
Riparian sweep	0.3 (0.3)	0.2 (0.1)	0.3 (0.2)
Taxa Richness			
Aquatic drift	15.5 (5.5)	14.1 (3.4)	15.6 (4.0)
Riparian sweep	19.5 (3.4)	18.3 (5.8)	18.5 (5.6)
Diversity			
Aquatic drift	2.1 (0.3)	1.8 (0.6)	1.7 (0.7)
Riparian sweep	2.2 (0.3)	2.2 (0.5)	2.3 (0.3)
Evenness			
Aquatic drift	0.79 (0.11)	0.70 (0.24)	0.64 (0.23)
Riparian sweep	0.75 (0.08)	0.76 (0.16)	0.79 (0.07)

1: counts were standardized by flow volume through drift nets

Based on the Bray-Curtis dissimilarity measure, there were no significant differences in the composition of aquatic invertebrate drift collections among Redside Dace status groups (MRPP observed delta = 0.70, $p = 0.43$). The bi-plot of non-metric dimensional scaling (nMDS) site-scores (Figure 4b) indicated a large degree of overlap among groups in multivariate space (stress value for 2-dimensional solution = 0.14). No taxa were considered as indicators of population status (Indicator Species Analysis; $p > 0.05$).

Canonical Correspondence Analysis (CCA) extracted three axes that explained 40.5% of the variation in aquatic invertebrate collections among sites. However, neither the model (p = 0.16) or correlations between taxa and instream habitat variables (p = 0.13) were significant.

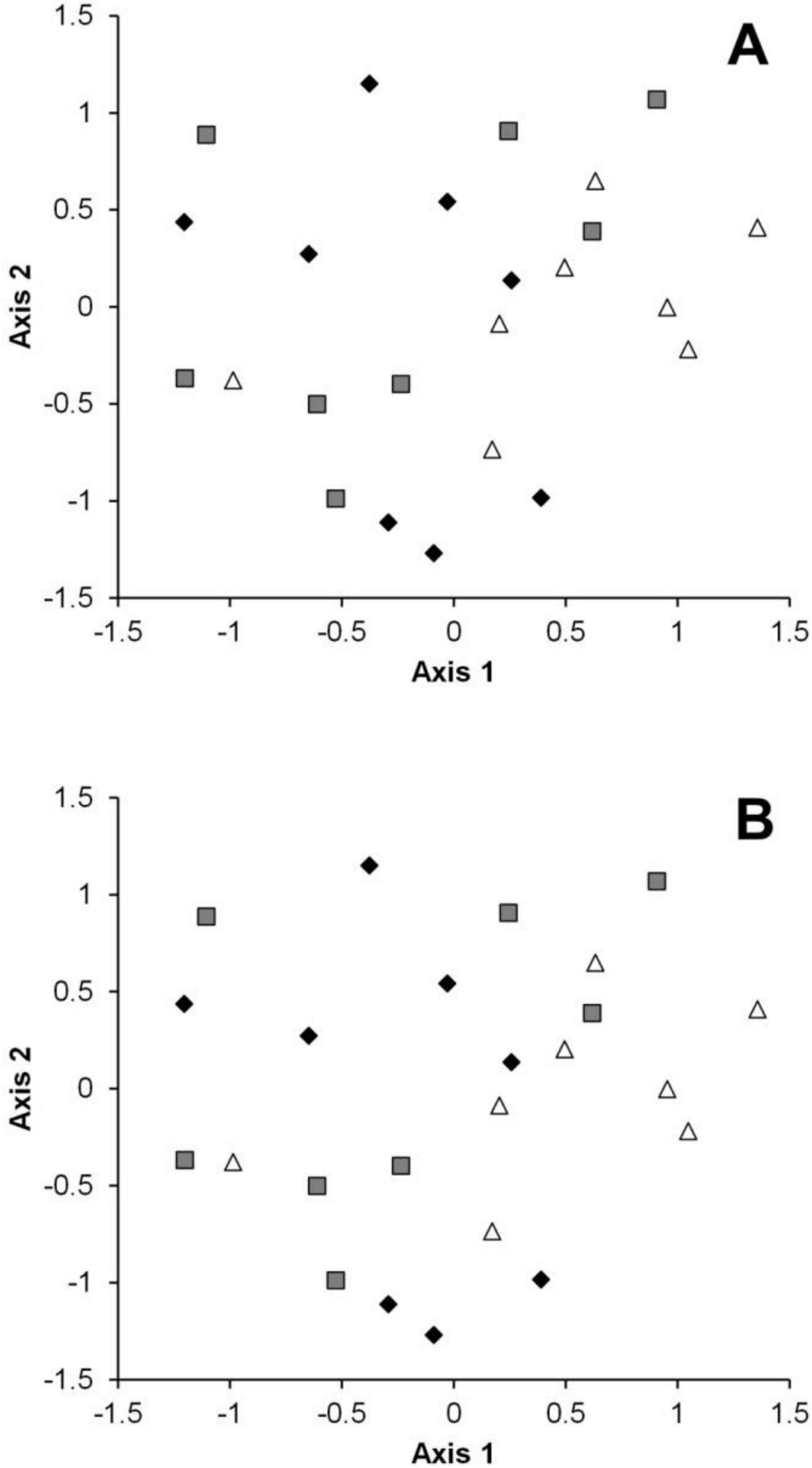


Figure 4. Bi-plots of taxa-based non-metric dimensional scaling (nMDS) site-scores (n=24) associated with three Redside Dace population status groups (stable Δ; declining ■; and extirpated ♦). Analysis was done for (A) terrestrial invertebrates and (B) aquatic invertebrates.

TERRESTRIAL INVERTEBRATES

Riparian sweeps netted a total of 3471 invertebrates that were identified to 91 families (or orders). Formicidae (19.4% of individuals collected), Cicadellidae (leaf hoppers, 18.2%) and Aranae (spiders, 8.0%) were the most abundant terrestrial invertebrates in riparian sweeps (Table 5, Figure 3a). The largest difference among Redside Dace population groups was the greater number of individuals netted from riparian vegetation at stable sites (Table 4). However, mean abundance and biomass of terrestrial invertebrates did not differ significantly among groups (ANOVA: abundance, F = 0.66, p = 0.43; biomass, F = 0.06, p = 0.81). Similarly, taxa diversity, richness or Pielou's evenness did not differ significantly among groups (ANOVA: F = 0.11, p = 0.75; F = 0.16, p = 0.69; F = 0.63, p = 0.44 respectively). Numbers of adult Diptera (important Redside Dace prey) netted from riparian vegetation at stable (mean = 30.8, standard deviation (SD) = 48.4), declining (mean = 34.9, SD = 25.6) and extirpated (mean = 34.3, SD = 48.4) sites were very similar. Surprisingly, Empidids (danceflies) were more widespread (detected at twice as many sites) and collected in considerably greater numbers from extirpated (mean = 18.1, SD = 39.2) and declining (mean = 4.9, SD = 9.8) sites than stable Redside Dace sites (mean = 0.75, SD = 1.0). However, these differences were not significant (ANOVA: F = 1.9, p = 0.17).

Based on the Bray-Curtis dissimilarity measure, there were no significant differences in the composition of terrestrial invertebrate collections among Redside Dace status groups (MRPP observed delta = 0.65, p = 0.20). The bi-plot of non-metric dimensional scaling (nMDS) site-scores (Figure 4a) indicated a large degree of overlap among groups in multivariate space (stress value for 2-dimensional solution = 0.17). Indicator species analyses identified that Acrididae (grasshopper) was associated with stable Redside Dace population sites (stat = 0.697, p = 0.03).

CCA extracted three axes that only explained 25.5% of the variation in terrestrial invertebrate collections among sites. Neither the model (p = 0.13) or correlations between taxa and riparian vegetation and bank stability variables (p = 0.11) were significant.

Table 5. Invertebrate taxa collected from riparian vegetation at 24 sites over a range of Redside Dace population status.

Class	Order	Taxa	Stable	Declining	Extirpated
Clitellata	Arhynchobdellida	Hirudinae		+	+
Arachnida	Aranae	Aranae	+	+	+
Arachnida	Trombidiformes	Hygrobatoidae	+		
Diplopoda	Spirobolida	Spirobolidae			+
Collembola	Entognatha	Collembola			+
Insecta	Blattodea	Blatellidae	+		
Insecta	Coleoptera	Cantharidae		+	+
Insecta	Coleoptera	Cerambycidae	+		
Insecta	Coleoptera	Chrysomelidae	+	+	+
Insecta	Coleoptera	Coccinellidae	+	+	+
Insecta	Coleoptera	Coleoptera		+	+
Insecta	Coleoptera	Colydiidae	+		+
Insecta	Coleoptera	Curculionidae	+	+	+
Insecta	Coleoptera	Elateridae	+	+	+
Insecta	Coleoptera	Lampyridae	+	+	
Insecta	Coleoptera	Mycetophagidae		+	
Insecta	Coleoptera	Rhysodidae		+	

Class	Order	Taxa	Stable	Declining	Extirpated
Insecta	Dermaptera	Forficulidae	+	+	+
Insecta	Diptera	Acartophthalmidae	+		
Insecta	Diptera	Anthomyiidae	+	+	
Insecta	Diptera	Asilidae		+	+
Insecta	Diptera	Asteiidae		+	
Insecta	Diptera	Brachycera	+		
Insecta	Diptera	Chironomidae	+	+	+
Insecta	Diptera	Chloropidae	+	+	+
Insecta	Diptera	Culicidae	+	+	+
Insecta	Diptera	Curtonotidae	+		
Insecta	Diptera	Diptera	+	+	+
Insecta	Diptera	Dolichopodidae	+	+	+
Insecta	Diptera	Drosophilidae	+	+	
Insecta	Diptera	Dryomyzidae		+	
Insecta	Diptera	Empididae	+	+	+
Insecta	Diptera	Ephydridae	+	+	
Insecta	Diptera	Heleomyzidae		+	+
Insecta	Diptera	Micropezidae	+	+	
Insecta	Diptera	Muscidae		+	+
Insecta	Diptera	Pallopteridae	+	+	+
Insecta	Diptera	Rhagionidae	+		
Insecta	Diptera	Scathophagidae	+	+	+
Insecta	Diptera	Sciomyzidae	+		
Insecta	Diptera	Simuliidae		+	+
Insecta	Diptera	Sphaeroceridae		+	
Insecta	Diptera	Syrphidae		+	+
Insecta	Diptera	Tachinidae	+		
Insecta	Diptera	Tephritidae	+		+
Insecta	Diptera	Therevidae		+	+
Insecta	Diptera	Tipulidae	+		+
Insecta	Ephemeroptera	Baetidae		+	
Insecta	Hemiptera	Cicadellidae	+	+	+
Insecta	Hemiptera	Cixiidae			+
Insecta	Hemiptera	Coreidae			+
Insecta	Hemiptera	Delphacidae			+
Insecta	Hemiptera	Hemiptera	+	+	+
Insecta	Hemiptera	Membracidae	+	+	+
Insecta	Hemiptera	Miridae	+	+	+
Insecta	Hemiptera	Nabidae	+		
Insecta	Hemiptera	Pentatomidae	+	+	
Insecta	Hemiptera	Reduviidae			+
Insecta	Hemiptera	Rhyparochromidae		+	
Insecta	Hemiptera	Saldidae	+	+	
Insecta	Hymenoptera	Cimbicidae	+		+
Insecta	Hymenoptera	Crabronidae		+	+
Insecta	Hymenoptera	Dryinidae	+	+	
Insecta	Hymenoptera	Formicidae	+	+	+
Insecta	Hymenoptera	Hymenoptera			+

Class	Order	Taxa	Stable	Declining	Extirpated
Insecta	Hymenoptera	Ichneumonidae	+	+	+
Insecta	Hymenoptera	Pelecinidae	+		
Insecta	Hymenoptera	Sphecidae		+	
Insecta	Hymenoptera	Symphta(sub-order)		+	
Insecta	Hymenoptera	Vespidae		+	
Insecta	Lepidoptera	Lepidoptera	+	+	+
Insecta	Lepidoptera	Papilionidae		+	
Insecta	Mantodea	Mantidae			+
Insecta	Neuroptera	Neuroptera			+
Insecta	Odonata	Calopterygidae		+	+
Insecta	Odonata	Coenagrionidae	+		
Insecta	Odonata	Lestidae			+
Insecta	Orthoptera	Acrididae	+	+	
Insecta	Orthoptera	Orthoptera	+		
Insecta	Orthoptera	Tetrigidae	+	+	
Insecta	Plecoptera	Capniidae	+		
Insecta	Plecoptera	Perlodidae		+	
Insecta	Plecoptera	Pteronarcyidae	+		
Insecta	Trichoptera	Hydropsychidae	+	+	
Malacostraca	Isopoda	Oniscidae	+	+	+
Gastropoda	Basommatophora	Physidae	+	+	+
Gastropoda	Basommatophora	Planorbidae	+	+	+
Gastropoda	Heterostopha	Valvatidae			+
Gastropoda	Heterostopha	Gastropoda(class)			+

DISCUSSION

In southern Ontario watersheds, negative associations between Redside Dace occurrence and population size and urbanization (*i.e.,* road density, or impervious land-cover) have been identified (Poos et al. 2012, Wallace et al. 2013). However, limited research documenting changes to Redside Dace habitat in response to urbanization has been undertaken. Common low-order stream channel responses include increased channel dimensions and decreased complexity of instream habitats (Miltner et al. 2004, O'Driscoll et al. 2010). These alterations are expected to reduce the amount and quality of pool and riffle habitats; both important to Redside Dace. Parish (2004) found GTA stream sites with Redside Dace to have steeper gradients, smaller channel widths, and smaller wetted width to depth ratios than those sites without Redside Dace. However, Reid et al. (2008) failed to identify expected differences in the amount of deep pool habitat and fine sediments between historical and currently occupied stream sites in the GTA. Alternatively, differences were limited to the amount of cover, riffles, and shallow pools.

In this study, instream habitat differences among Redside Dace population status groups were not statistically significant. While local and upstream urban-cover contrasted among the groups of sites sampled, the influence of landcover on stream habitat condition (and on stream invertebrates) can be confounded by surficial geology and topography in an area (Van Sickle 2003, Stanfield and Kilgour 2006, Stanfield and Kilgour 2012). Across the study area, surficial geology varies with either Paleozoic bedrock, fine- and coarse-textured glaciolacustrine deposits or till (stony or stone-poor) represented at sampling sites (Ontario Geological Survey

2012). It is not known how much variation in habitat condition among sites can be explained by local surficial geology, local and upstream urban landcover, or historical agricultural land-use. For some habitat measures relevant to Redside Dace, the OSAP rapid assessment methodology may not be suitable for detecting differences among sites (Reid et al. 2008). The substrate class “fines” includes all < 2 mm diameter particles. Therefore, it is not possible to distinguish clean, coarse sand from deposits of silts and clay. Pools between 101 and 600 mm are classified as deep. This range is probably too broad for small streams where pools are rarely deeper than 600 mm. Habitat classification is also informed by hydraulic head measurements. As habitat assessments were completed at base-flow levels, a lack of current likely inflated estimates of pool habitat because uniform glides or run habitats are misclassified. Lastly, the statistical power of this study’s design to detect habitat differences was likely low. Sample sizes within each population group (*i.e.,* n = 8) were relatively small, and variation around mean values was large. Low power also likely affected the interpretation of differences for other measures, such as aquatic and terrestrial invertebrates.

Riparian zones are an important component of critical habitat for stream-dwelling fishes at risk (Richardson et al. 2010). For Redside Dace, overhanging stream vegetation stabilizes streambanks, promotes the production of terrestrial insects (a primary diet item), provides cover, and maintains cooler summer water temperatures (Daniels and Wisniewski 1994; Novinger and Coon 2000). In this study, stable Redside Dace population sites in the GTA were characterized as having abundant riparian grass cover and an open tree canopy. In comparison, greater amounts of bare ground and overhead tree cover were present at sites where populations are in decline, or have been lost. This result is in agreement with Andersen (2002), who observed Redside Dace distribution changes in the Lynde Creek watershed (Whitby, Ontario) to coincide with changes in riparian cover (*i.e.,* shift from grasses and shrubs to cedar *Thuja occidentalis* forest). Protection and rehabilitation goals for Ontario Redside Dace populations include rehabilitating degraded habitats in areas adjacent to occupied reaches (RDRT 2010). Results from this study indicate the importance of re-establishing abundant riparian grass cover at identified degraded sites.

Redside Dace is a surface and above-surface feeder with a diet dominated by terrestrial invertebrates. Adult Diptera often represent a large component of prey consumed, while aquatic drifting invertebrates are much less frequently consumed (Schwartz and Norvell 1958, McKee and Parker 1982, Daniels and Wisniewski 1994). Diptera were ubiquitous across GTA study sites, not identified as indicator taxa, and similarly abundant across Redside Dace population status categories. Diptera are a heterogeneous group that have shown conflicting relationships with environmental factors at multiple scales that could mask any significant differences (Malmqvist and Hoffsten 2000). Empidids (danceflies) have been described to be an important diet item during the summer (Schwartz and Norvell 1958, Daniels and Wisniewski 1994). Surprisingly, danceflies were rarely collected from riparian vegetation at stable Redside Dace population sites. The paucity of danceflies might indicate that sweep nets were not effective for collecting adult danceflies at these sites. Emergence traps are a more common method for collecting danceflies (Harper 1980, Ivković et al. 2012, Cadmus et al. 2016) and should be considered as an alternate method for future Redside Dace prey research. Alternatively, the responses of Redside Dace and danceflies to environmental stressors and habitat variation across GTA streams may simply differ (Johnson and Ringler 2014). For example, low numbers of empidids could reflect non-targeted effects of insecticide application in agricultural areas (Naranjo et al. 2004, El-Wakeil and Volkmer 2013).

Although surrounding and upstream urban landcover varied greatly among the study sites, multivariate differences in the composition of aquatic or terrestrial invertebrate assemblages were not detected in this study. Only, one taxon (Acrididae, grasshopper) was identified (indicator species analysis) to be strongly associated with any group of Redside Dace sites. Given the relatively high coverage of riparian grasses at stable sites, this association is not surprising. Other taxa were neither associated with groups of sites, or with habitat variables. These results may reflect the similarity of instream habitat among sampled sites, or the differing responses of Redside Dace and of aquatic and terrestrial invertebrates to environmental conditions (Johnson and Ringler 2014). In contrast to this study, other recent assessments of urbanization impacts on invertebrates in Lake Ontario tributaries identified differences in diversity and assemblage composition (Stanfield and Kilgour 2006, Wallace et al. 2013). Contrasting results may reflect study design differences. Past studies used qualitatively different invertebrate data (benthic invertebrates collected by kick and sweep methods), and analyzed much larger datasets (>100 sites).

Mapping and monitoring of Redside Dace habitat are priority recovery actions (RDRT 2010). These actions inform management activities such as the review of development and instream work proposals, population status assessments, and the planning of rehabilitation projects. Habitat sampling methods used in this study were successful at discriminating sites based on riparian vegetation, and therefore, would be suitable for future riparian health assessments. However, components of the OSAP rapid habitat assessment methodology that characterize stream bed material and availability of different instream habitat types (i.e., riffles or pools) were not similarly effective (also Reid et al. 2008). Given the importance of pools to Redside Dace, it is recommended that suitability of field methods for quantifying pool volume and depth, and fine sediment accumulation (see Hilton and Lisle 1993) be assessed.

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