An environmental DNA-based survey for Redside Dace (*Clinostomus elongatus*) in Greater Toronto Area watersheds

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ABSTRACT

Redside Dace (*Clinostomus elongatus*) is a small, colorful cyprinid found in the pools and slow-flowing sections of small Ontario streams. As a result of recent declines and threats to remaining populations, the species has been assessed by COSEWIC as Endangered in Canada. Ongoing inventory efforts are important to delineate areas of protected habitat and monitor trends in the distribution and abundance of Redside Dace populations. Between 2013 and 2015, we collected water samples (five 1-litre samples) from 149 sites in nine Greater Toronto Area watersheds (Bronte Creek, East Morrison Creek, Cold Creek, Kettleby Creek, Lynde Creek, Rainbow Creek, Sharon Creek, Spencer Creek, and Urfe Creek). The presence of Redside Dace at each site was assessed using environmental DNA (eDNA) methods based on species-specific quantitative polymerase chain reaction (qPCR) primers and probe. Only five of the sites sampled for Redside Dace yielded positive eDNA detections. Four positive detections were from Lynde Creek, and one positive detection was from East Morrison Creek. Single-pass backpack electrofishing surveys failed to collect Redside Dace from Lynde and East Morrison creeks. Study results support earlier assessments of declining population status for Redside Dace in these watersheds.

RÉSUMÉ

Le méné long (*Clinostomus elongatus*) est un petit cyprinidé coloré que l'on retrouve dans les fosses et les sections à faible courant des petits cours d'eau de l'Ontario. En raison du déclin récent des populations et des menaces pesant sur celles qui restent, l'espèce a été classée par le COSEPAC comme étant en voie de disparition au Canada. Les efforts continus pour évaluer les stocks sont importants pour délimiter les zones d'habitat protégé et faire le suivi des tendances de la répartition et de l'abondance des populations de méné long. Entre 2013 et 2015, nous avons recueilli des échantillons d'eau (cinq échantillons d'un litre chacun) à 149 sites dans 9 bassins hydrographiques de la région du Grand Toronto (ruisseaux Bronte, East Morrison, Cold, Kettleby, Lynde, Rainbow, Sharon, Spencer et Urfe). La présence du méné long à chaque site a été évaluée au moyen de méthodes de prélèvement d'ADN environnemental (ADNe) fondées sur des amorces et des sondes conçues pour déterminer la réaction en chaîne de la polymérase quantitative (RCPq) propres à l'espèce. La présence d'ADNe du méné long a été détectée à seulement cinq des sites échantillonnés. Quatre de ces détections positives se sont produites dans le ruisseau Lynde et l'autre, dans le ruisseau East Morrison. Les relevés effectués au moyen d'un dispositif portatif de pêche à l'électricité n'ont pas permis de recueillir de ménés longs dans les ruisseaux Lynde et East Morrison. Les résultats de l'étude confirment les évaluations ultérieures : les populations de méné long sont à la baisse dans ces bassins hydrographiques.

INTRODUCTION

The Redside Dace (*Clinostomus elongatus*) is a small, colorful cyprinid found in the pools and slow-flowing sections of small streams with moderate gradient and riffle–pool sequences (McKee and Parker 1982; Novinger and Coon 2000). As a result of recent declines and threats to remaining populations, the species has been assessed as Endangered in the province of Ontario (MNRF 2016) and in Canada (COSEWIC 2007). Redside Dace is thought to be extirpated from 10 of 24 Ontario watersheds, with eight of the remaining 14 populations in decline (COSEWIC 2007). Habitat degradation caused by urban development activities is considered one of the primary threats facing Canadian populations (COSEWIC 2007). Almost 80 percent of its Canadian distribution is located within the boundaries of the City of Toronto and adjacent municipalities (the Greater Toronto Area). By 2031, the human population in this region is projected to grow by 37% (MPIR 2013) and, accordingly, stressors associated with urban development are expected to increase.

The provincial government's goal for recovery of Redside Dace is to protect existing populations and their habitats and, where feasible, restore degraded habitats to allow for increased distribution adjacent to occupied reaches (MNRF 2010). A key action to support this goal has been the development of a regulation to protect Redside Dace habitat. The regulation defines Redside Dace habitat as, *any part of a stream or other watercourse that was used by a redside dace at any time during the previous 20 years and that provides suitable conditions for a redside dace to carry out its life processes*. Implementation of the regulation is supported by the following priority recovery actions:

- maintain a database of Redside Dace distribution and ensure that information on the currently occupied range of the species is available to appropriate planning authorities; and,
- establish a long-term monitoring program to continue to determine the distribution, abundance, and associated trends of Redside Dace and its habitats.

To date, the inventory and monitoring of Redside Dace streams have been done using either a bag seine or backpack electrofisher (Reid et al. 2008; Poos et al. 2012). Detection of environmental DNA (eDNA) from water samples is increasingly being used to document potential species occurrences in aquatic systems. It has successfully been validated over a wide range of aquatic organisms including fishes (Jerde et al. 2011), amphibians (Dejean et al. 2012), and plants (Scriver et al. 2015). As a result of reduced costs, non-intrusive sampling, and higher sensitivity compared to conventional methods (Ficetola et al. 2008; Darling and Mahon 2011), eDNA is gaining popularity as a monitoring tool for both invasive and endangered species (Ficetola et al. 2008; Wilcox et al. 2013; Moyer et al. 2014). Recently, an eDNA-based Redside Dace detection method was developed and validated by Serrao (2016).

In this study, we used eDNA methods to survey nine watersheds in the Greater Toronto area for Redside Dace. Except for Lynde Creek, reaches sampled were identified based on the need to update occurrence data supporting the Redside Dace habitat regulation. Without new Redside Dace detections, these reaches would be removed from the list of regulated areas, or the designation would change from occupied to recovery. Additionally, the eDNA based inventory was undertaken to inform the COSEWIC (Committee on the Status of Endangered Wildlife in Canada) status update assessment currently underway.

METHODS

The methods used for eDNA surveys by the Ontario Ministry of Natural Resources and Forestry (OMNRF), from field sampling and processing of environmental water samples to testing and validation of DNA surveillance results are summarized by Wilson et al. (2014).

During 2013, 2014, and 2015 we collected water samples from 149 sites in nine watersheds. Sampled watersheds in the Lake Ontario drainage include: Bronte Creek, Morrison Creek, Cold Creek (Humber River), Lynde Creek, Rainbow Creek (Humber River), Spencer Creek, and Urfe Creek. Kettleby and Sharon creeks (Holland River) are part of the Lake Simcoe drainage. The distribution of sampling sites within each watershed is presented in Figures 1 to 5. Sample collection occurred from mid-May to mid-October, when water temperatures were typically greater than 10°C. Sampling details and habitat characteristics are presented in Table 1.

At each site, five temporally replicated 1-litre water samples were collected from surface water. Individual samples were collected over a 1 hour time period, each separated by 15 minutes. Samples were stored in a cooler with ice-packs to prevent DNA degradation. A field control water sample was collected at the beginning of each day from the Otonabee River and stored in the cooler. Redside Dace is not present in the Otonabee River (Mandrak and Crossman 1992). Samples were stored at 4 ^{o}C overnight and filtered the following day.

All samples were filtered in the lab within 24 hours of collection using 47 mm Whatman 1.2 µm pore GF/C filters. DNA was extracted using the MoBio PowerWater kit® DNA Isolation Kit (http://www.mobio.com), and quantitative polymerase chain reaction (qPCR) amplified with species-specific primers and probe (Serrao 2016). The primer and probe set targeted an 83 base pair segment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. The COI gene is well suited for eDNA detection as there are species-level diagnostic differences between most species and a rapidly growing database of COI DNA barcodes is available (Hebert et al. 2003; Ratnasingham and Hebert 2007).

For each sample, three replicate qPCR reactions were run. The Redside Dace positive detection threshold was three DNA copies per qPCR reaction (Serrao 2016). Using this method, the probability of detecting Redside Dace during the spring and fall is greater than 0.6 (Serrao 2016). Mitochondrial DNA from samples with positive eDNA detections was sequenced at the COI gene to confirm species identity.

In addition to eDNA sampling, backpack electrofishing surveys were also completed at Morrison Creek and Lynde Creek sampling sites. Electrofishing was done following the Ontario Stream Assessment Protocol (Stanfield 2010). Lynde Creek electrofishing was done in partnership with the Central Lake Ontario Conservation Authority (CLOCA). Forty-seven Lynde Creek sites were electrofished during July 2014. Five Morrison Creek sites were electro-fished on July 23, 2015. At each site, 40 m of stream habitat was sampled. The four other sites sampled with eDNA methods in the spring were dry.

RESULTS AND DISCUSSION

Only five of the 149 sites sampled for Redside Dace yielded positive eDNA detections (Figures 1 to 5). Four positive detections were from the central portion of the Lynde Creek watershed (Figure 3) and a single positive detection was from the east branch of Morrison Creek. COI sequence confirmation of species identity was successful for two of the Lynde Creek detections and the Morrison Creek detection. Confirmation yielded 100% query coverage and 100% similarity to Redside Dace (Genbank Accession Numbers: JN024974.1, JN024973.1,

JN024972.1, JN024971.1, and EU524485.1). Other samples could not be confirmed due to poor PCR amplification. No Redside Dace were collected during backpack electrofishing of Lynde Creek and Morrison Creek sites.

Fourteen species were collected during electrofishing surveys of Lynde Creek sites in 2014. The most commonly encountered species were Blacknose Dace (*Rhinichthys atratulus*), Creek Chub (*Semotilus atromaculatus*), Fathead Minnow (*Pimephales promelas*), Johnny Darter (*Etheostoma nigrum*), Mottled Sculpin (*Cottus bairdii*), Rainbow Darter (*Etheostoma caerleum*), Rainbow Trout (*Oncorhynkus mykiss*), and White Sucker (*Catostomus commersonii*). The locations of positive eDNA-based detections in Lynde Creek contrasts with the survey by Anderson (2002), where Redside Dace were largely limited to the upper parts of the watershed. While Redside Dace have been recently (2013) collected from lower reaches of Lynde Creek (Reid unpublished data), the pattern of detections may also reflect downstream transport of DNA from upstream sources (Deiner and Altermatt 2014).

Species detected by electrofishing in Morrison Creek were Blacknose Dace, Creek Chub, and Fathead Minnow. Previously widespread throughout the watershed, the last detection of Redside Dace from Morrison Creek occurred in 2000 (COSEWIC 2007). Electrofishing and eDNA results support the previous COSEWIC assessment of a declining trend in range for the Morrison Creek population (Table 1 in COSEWIC 2007).

There were no positive detections from eDNA samples collected from Bronte Creek, Cold Creek, Kettleby Creek, Rainbow Creek, Sharon Creek, Spencer Creek, and Urfe Creek (Figures 1, 2, 4 and 5). The lack of detections is consistent with the catch records that indicate declines in each watershed, and no recent catches at many of the sites sampled (i.e. ~20 years since last catch at each location; Table 1 in COSEWIC 2007). As reported for other methods used to collect Ontario fishes at risk (see Dextrase et al. 2014), eDNA-based detection of Redside Dace will be imperfect. Greater certainty regarding the occupancy status of stream reaches can only be achieved by undertaking multiple repeat surveys.

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Table 1. Sampling details and habitat characteristics for nine Greater Toronto area watercourses sampled with eDNA methods. Median values for habitat characteristics (with ranges in parentheses) are provided.

Watercourse	Sites	Year	Sampling Dates	Water Temp. (^{o}C)	Conductivity (µs/cm)	Channel Width (m)	Water Depth (m)
Bronte Cr.	17	2014	June 4 to 10	16.5 (12.2 - 22.5)	788 (630 - 1350)	4.7 (0.9 - 12.7)	0.3 (0.1 - 0.7)
East Morrison Cr.	9	2015	May 20 to June 3	14.7 (13.3 - 19.8)	1600 (680 - 3172)	2.1 (1.1 - 2.8)	0.08 (0.05 - 0.2)
Cold Cr.	6	2014	June 10	14.5 (13.6 - 15.5)	532 (526 - 577)	4.7 (3.4 - 6.5)	0.24 (0 - 0.5)
Kettleby Cr.	11	2014	June 3 to 23	16.1 (12.7 - 22)	796 (289 - 1878)	2.2 (0.8 – 6.0)	0.17 (0.03 - 0.5)
Lynde Cr.	48	2013	September 17 to October 3	12.2 (7.5 - 17.9)	568 (269 - 1696)	2.6 (0.4 - 9.2)	0.15 (0.02 - 0.6)
	24	2014	July 2 to 17	17.4 (11.5 - 23.8)	639 (516 - 1497)	1.5 (0.5 - 7.1)	0.17 (0.03 - 0.9)
Rainbow Cr.	5	2015	October 6	15.5 (14.5 - 16.5)	1511 (1285 - 1580)	3.6 (2.5 - 5.5)	0.09 (0.03 - 0.2)
Sharon Cr.	3	2014	June 9	16.8 (13.2 - 18.2)	1615 (1182 - 2213)	1.2 (1.2 - 1.8)	0.1 (0.03 - 0.4)
	6	2015	October 8	12.7 (8.8 – 15.0)	2185 (1117 - 3540)	1.3 (0.8 - 4.7)	0.12 (0.03 - 0.9)
Spencer Cr.	11	2015	June 3 to 16	18.6 (13.6 - 22.7)	602 (489 - 1013)	8.5 (2.9 - 13.5)	0.4 (0.05 - 1.2)
Urfe Cr.	9	2015	May 14 to June 29	17.4 (10.1 - 20.9)	635 (526 - 867)	2.3 (0.9 - 7.4)	0.2 (0.02 - 0.5)

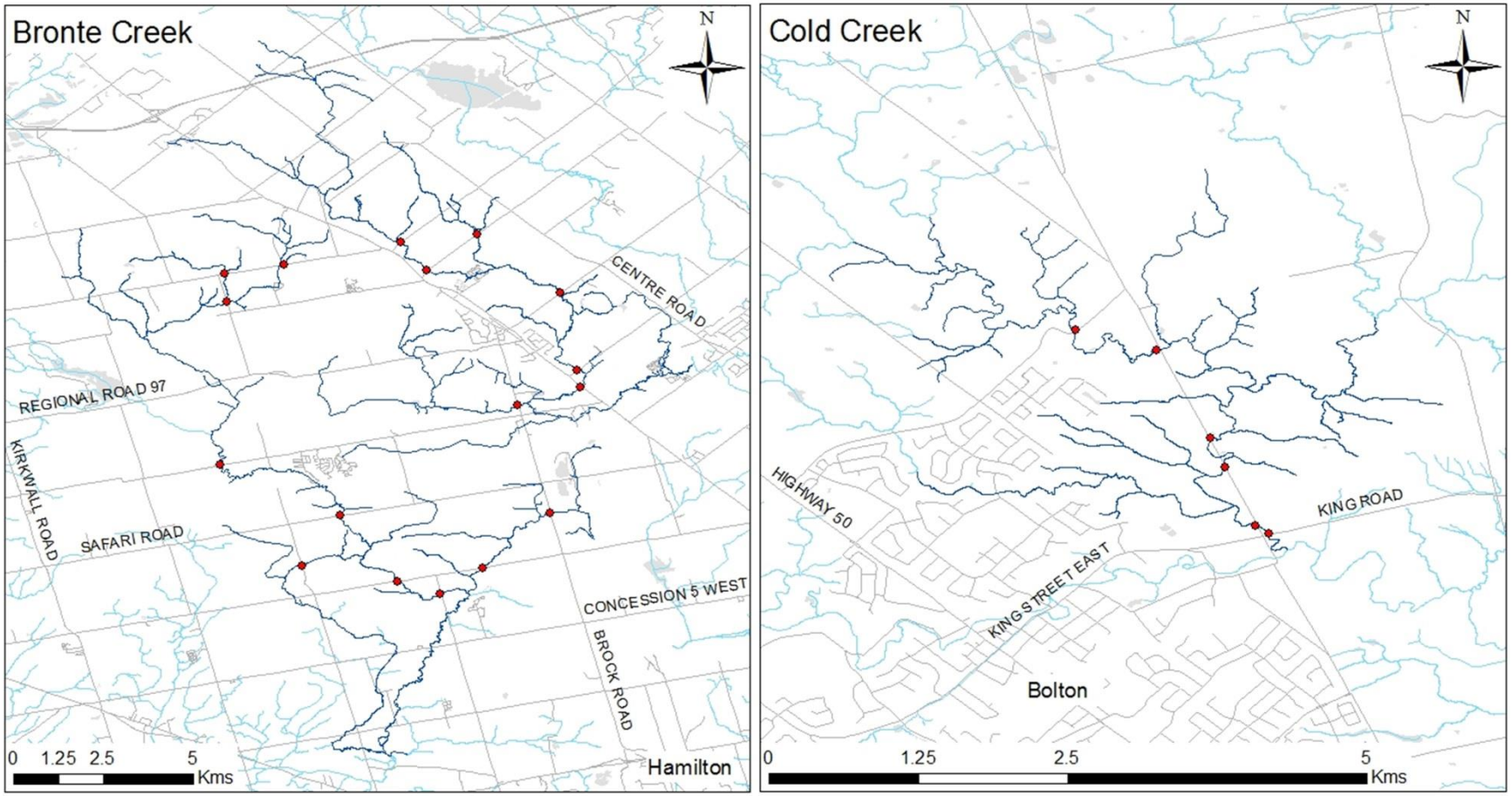

Figure 1. Distribution of Redside Dace eDNA sampling sites in Bronte Creek (n=17) and Cold Creek (Humber River watershed) (n=6). Five 1-L water samples were collected at each site.

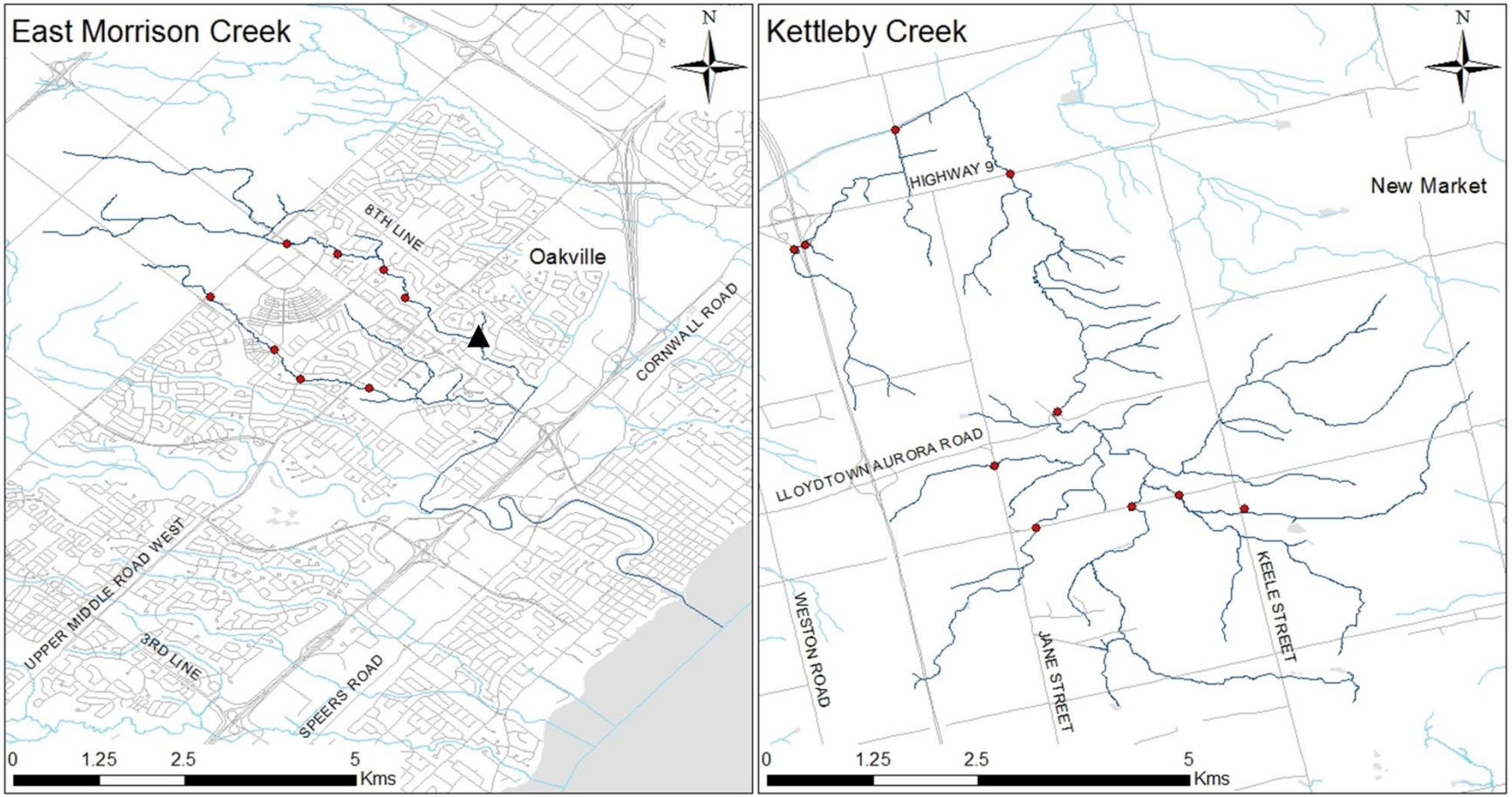

Figure 2. Distribution of Redside Dace eDNA sampling sites in East Morrison (n=9) and Kettleby (n=11) creeks. Five 1-L water samples were collected at each site. The locations of positive eDNA-based detections are identified (▲).

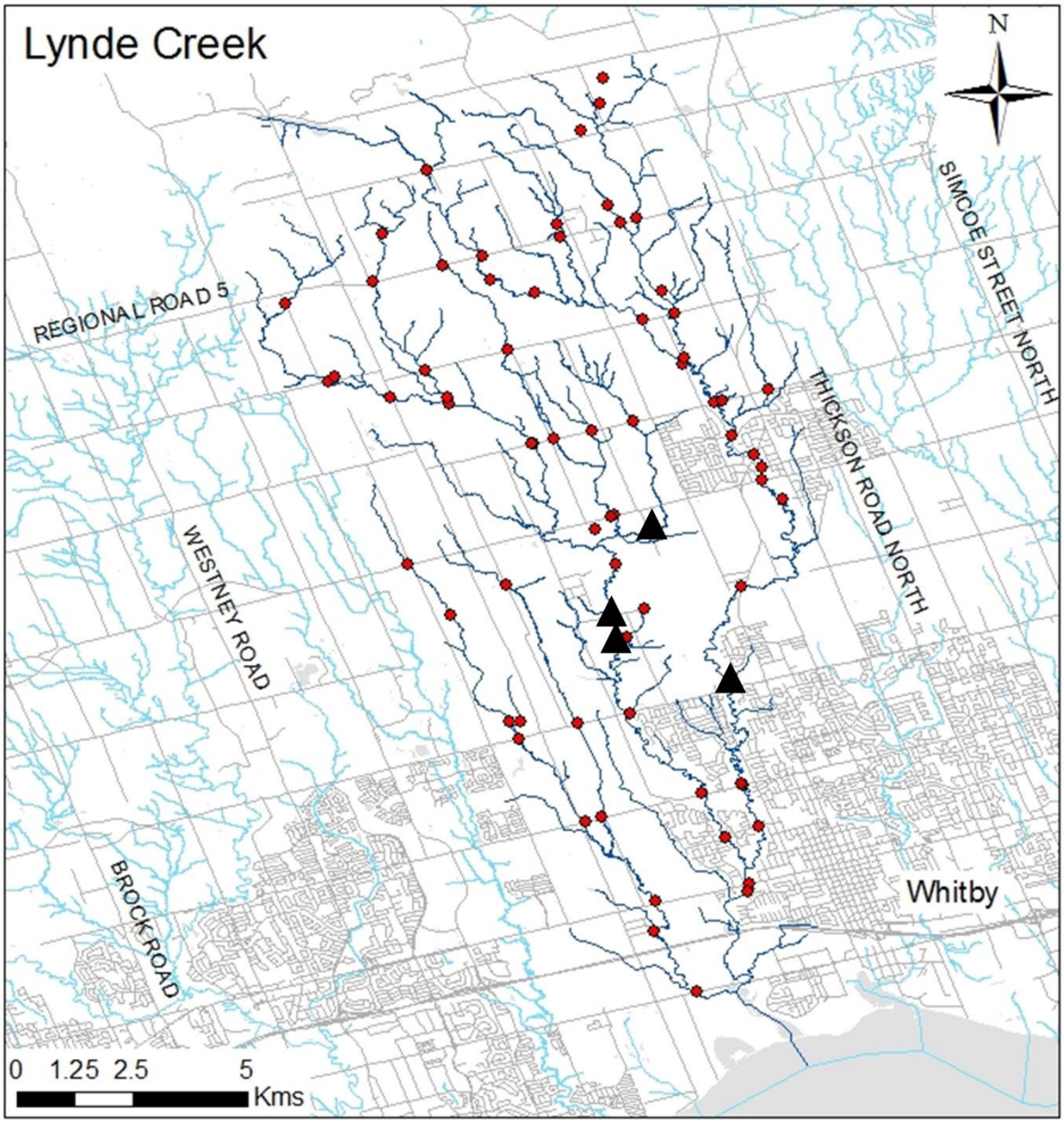

Figure 3. Distribution of Redside Dace eDNA sampling sites in Lynde Creek (n=72). Five 1-L water samples were collected at each site. The locations of positive eDNA-based detections are identified (▲).

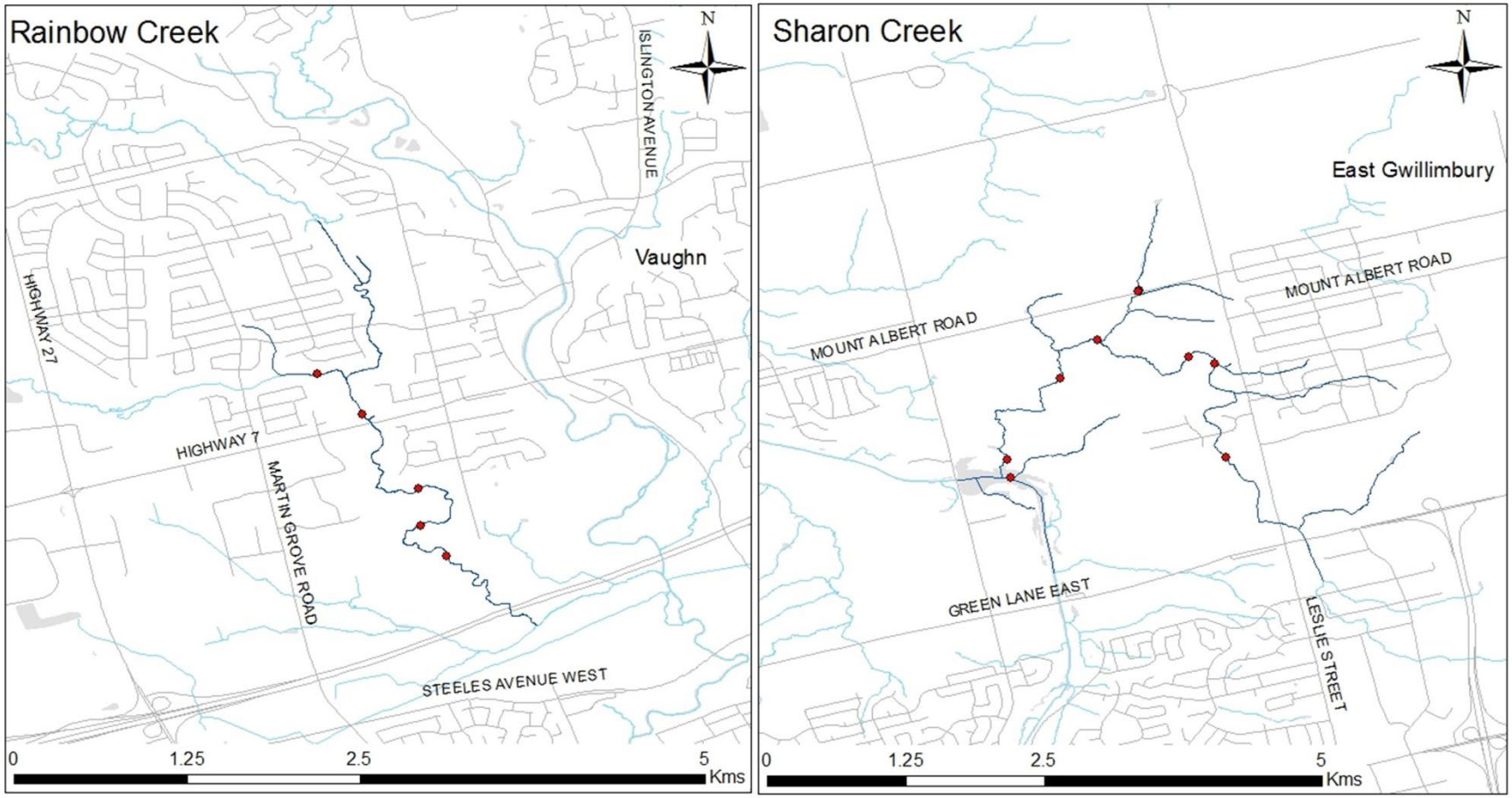

Figure 4. Distribution of Redside Dace eDNA sampling sites in Rainbow (n=5) and Sharon (n=9) creeks. Five 1-L water samples were collected at each site.

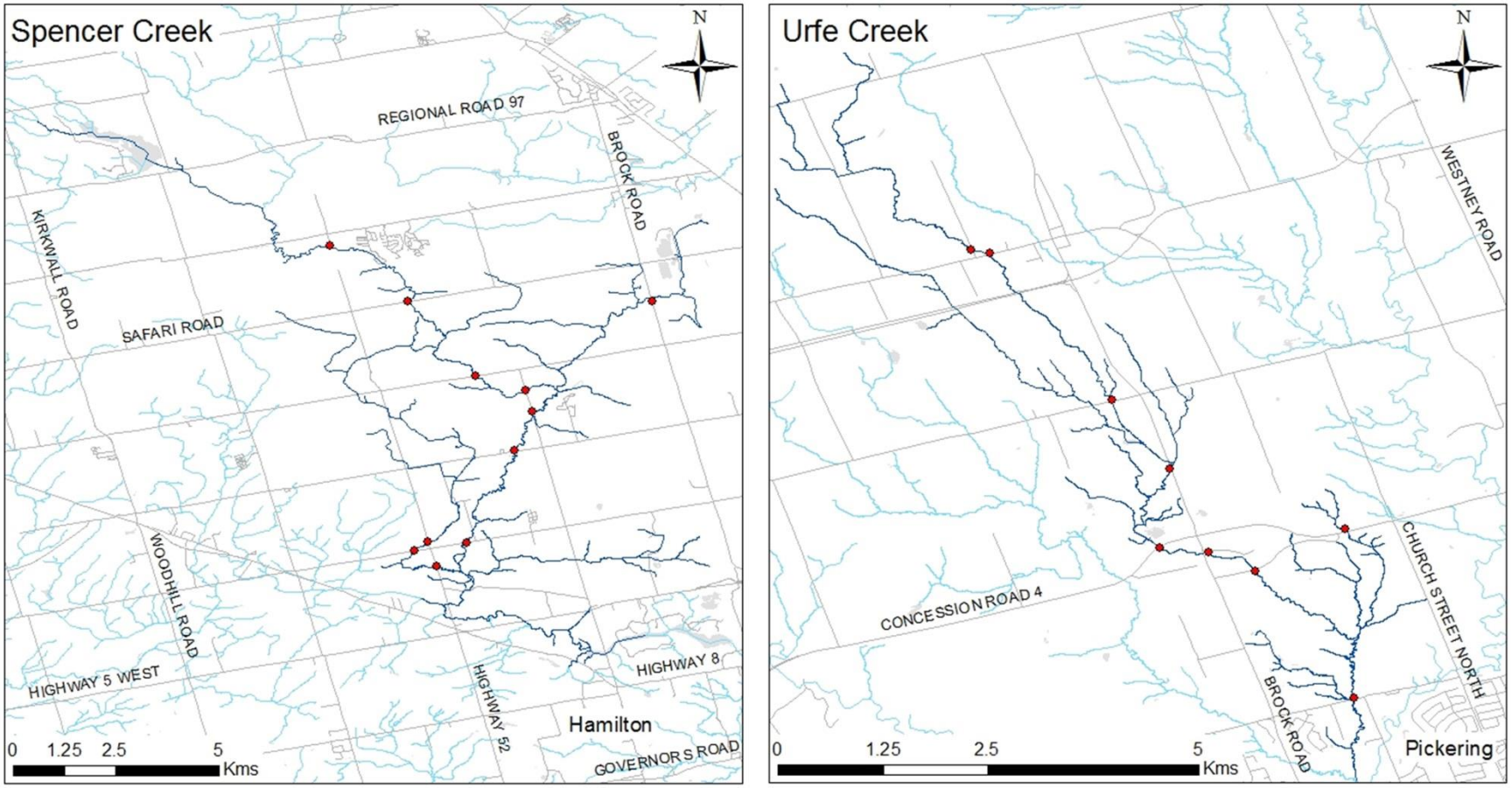

Figure 5. Distribution of Redside Dace eDNA sampling sites in Spencer (n=11) and Urfe (n=9) creeks. Five 1-L water samples were collected at each site.